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Medullary Thyroid Cancer: Diagnosis and Management

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Introduction

Medullary thyroid carcinoma is a well-differentiated thyroid tumor maintaining the biochemical and pathological features of the parafollicular or calcitonin-producing C cells from which it derives [1,2]. Its origin makes it a separate entity from the other differentiated thyroid carcinomas.

The overall frequency of medullary thyroid carcinoma is not well established, while its prevalence is 5–10% in all thyroid malignancies, 0.4–1.4% in all thyroid nodules, and less than 1% in the thyroids of subjects submitted to autopsy. Contrary to papillary and follicular carcinomas, no difference in distribution between females and males is observed. The clinical appearance is mainly in the fourth and fifth decades, but a wide range of age at onset is present [3–6].

No significant environmental factors or ethnic differences associated with the development of medullary thyroid carcinoma have been identified, although associations with prior thyroid diseases and other disorders such as hypertension, allergies, and gallbladder disease have been reported in a pooled analysis of epidemiological studies [7].

The pathogenetic mechanism has been recognized in the activation of the *RET* proto-oncogene [8–10]. According to the somatic or germline localization of the activated *RET* oncogene, two different forms are recognized: the sporadic form, which accounts for about 75% of cases, and the hereditary or familial form, which accounts for the remaining 25%. Only the hereditary form affects children and, generally, the most aggressive is the multiple endocrine neoplasia type 2B (MEN2B) syndrome in the clinically affected child [11–13].

The biological behavior of medullary thyroid carcinoma is much less favorable when compared with that of the other well-differentiated thyroid carcinomas even though it is not as unfavorable as that of anaplastic carcinoma [14] (Figure 21.1). A 10-year survival of about 50% of medullary thyroid carcinoma patients is reported in several series. Both the cure and survival of these patients are positively affected by an early diagnosis [15,16].

Clinical Presentation

Sporadic Form

The most common clinical presentation of sporadic medullary thyroid carcinoma is a thyroid nodule, either single or belonging to a series of nodules configuring the clinical picture of a multinodular goiter. With the exception of the simultaneous presence of diarrhea and/or flushing syndrome, which is however rare and usually related to an advanced metastatic disease, patients do not generally have any specific symptom. The association of thyroid nodular disease with a lump in the neck may

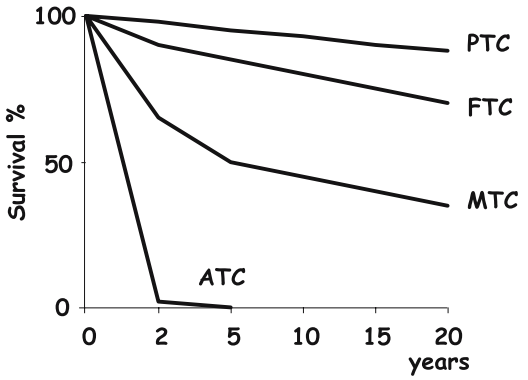

Figure 21.1 Survival rate of patients affected by different histotypes of thyroid carcinoma. MTC patients have a 10-year survival rate of about 50%, which is lower than that of patients with papillary and follicular thyroid carcinoma but higher than that of patients with anaplastic thyroid carcinoma.

lead the clinician to suspect a thyroid malignancy but not specifically a medullary thyroid carcinoma.

Hereditary Forms

In about 25% of cases the medullary thyroid carcinoma is one of the components of the multiple endocrine neoplasia type 2 syndrome, which is an autosomal dominant inherited syndrome with a variable degree of expressivity and an age-related penetrance. As shown in Table 21.1, three different hereditary syndromes can be classified according to the involved organs: (a) multiple endocrine neoplasia type 2A (MEN2A), a syndrome consisting of medullary thyroid carcinoma, pheochromocytoma, and parathyroid neoplasia [17]; (b) MEN2B, a syndrome consisting of medullary thyroid carcinoma, pheochromocytoma, mucosal neuromas, and ganglioneuromatosis [18]; (c) familial medullary thyroid carcinoma (FMTC), which is characterized by the presence of an inheritable medullary thyroid carcinoma with no apparent association with other endocrine neoplasia [19]. After the introduction of *RET* genetic screening, the relative prevalence of the FMTC syndrome has been found to be much higher (from 10% to 50% of all MEN syndromes) (Figure 21.2). The increased number of FMTC cases is mainly due to the high number of apparently sporadic medullary thyroid carcinomas demonstrated to be familial cases by the *RET* mutation analysis [20,21].

Table 21.1 Prevalence of different endocrine neoplasia and other clinical manifestations in MEN2 syndromes

	Clinical manifestation	Prevalence (%)
MEN2A	Medullary thyroid carcinoma	100
	Pheochromocytoma	50
	Parathyroid adenomas	10–30
	Cutaneous lichen amyloidosis	<10
MEN2B	Medullary thyroid carcinoma	100
	Mucosal neuromas (tongue, subconjunctivas)	100
	Ganglioneuromatosis	100
	Marfanoid habitus	65
	Pheochromocytoma	45
FMTC	Medullary thyroid carcinoma	100

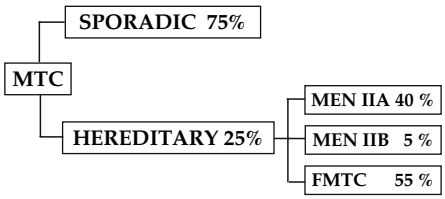

Figure 21.2 Classification and relative prevalence of different forms of medullary thyroid carcinoma according to *RET* genetic screening and clinical manifestations.

The clinical appearance of medullary thyroid carcinoma in MEN syndromes is that of a thyroid nodular disease, similar to that of the sporadic form with the exception that it is usually bilateral, multicentric, and almost invariably associated with C-cell hyperplasia [22–24]. The clinical course of the medullary thyroid carcinoma varies considerably in the three syndromes. It is very aggressive and almost invariably unfavorable in MEN2B, with affected patients rarely surviving after adolescence. It is most indolent in the majority of patients with the FMTC form and shows variable degrees of aggressiveness in patients with MEN2A. Different types of *RET* gene mutations account for different biological behavior [25–28] and separate therapeutic protocols have been defined for the treatment of medullary thyroid carcinoma occurring in the three different syndromes [29].

Between 10% and 30% of patients with MEN2A develop hyperparathyroidism during the third to fourth decades of life. The clinical findings are superimposable on those of the sporadic form of hyperparathyroidism and very often no specific symptoms are present. At variance with the sporadic form, multiple parathyroid hyperplasia or adenomatosis is most commonly found [17,30]. Hyperparathyroidism has only occasionally been reported in patients with MEN2B [18].

About 50% of MEN2A and 40–45% of MEN2B patients develop pheochromocytoma, which shares the same characteristics in both syndromes. Contrary to the sporadic form of pheochromocytoma, the adrenal tumors of MEN syndromes are usually bilateral and multicentric. However, the two adrenal glands are rarely simultaneously involved and a mean period of 10 years usually elapses between the development of the tumor in the two glands.

The MEN2B syndrome is characterized by the association with mucosal neuromas, which are mainly located on the distal tongue and subconjunctival areas, and ganglioneuromatosis affecting the gastrointestinal tract. MEN2B patients may be easily recognized on physical examination by the typical marfanoid habitus characterized by thin and inappropriately long extremities and pectus excavatum [18,31–33]. Thick lips and eyelids are frequently observed in the presence of mucosal neuromas, and are usually clearly evident when eyes and mouth are explored (Figure 21.3). Gastrointestinal disorders due to the intestinal neuromas throughout the intestinal tract, including obstructive symptoms, cramping and diarrhea, are frequently observed in early childhood.

An association with cutaneous lichen amyloidosis (CLA), a characteristically a pigmented and itchy skin lesion specifically localized in the interscapular region of the back (Figure 21.4),

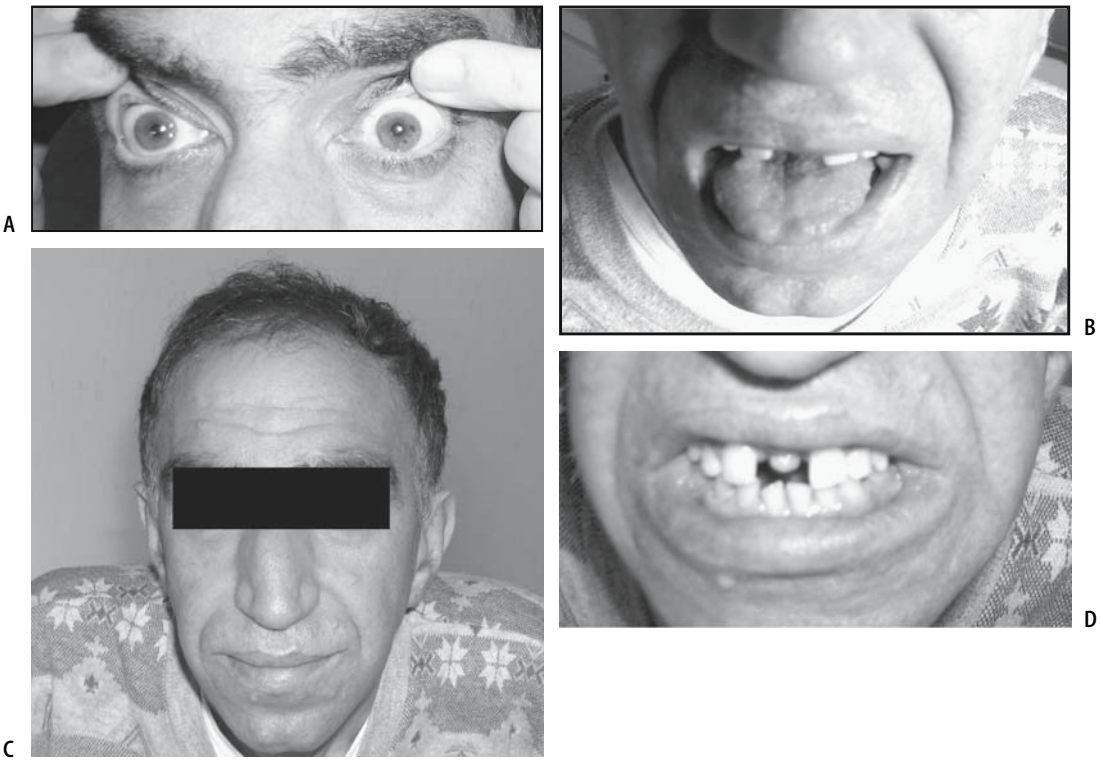

Figure 21.3 Clinical features of MEN2B syndrome. **A** Characteristic mucosal neurinomas of subconjunctivas are apparent. **B** Characteristic mucosal neurinomas of the distal part of the tongue are apparent. **C** Characteristic thick lips and marfanoid habitus. **D** Thick lips and mucosal neurinomas.

Figure 21.4 Cutaneous lichen amyloidosis (CLA) in a patient affected by MEN2A. The figure shows the characteristic location in the interscapular regions.

has been reported in less than 10% of MEN2A families [34,35]. The development of CLA may precede the development of the medullary thyroid carcinoma: thus, when present it is almost invariably diagnostic of MEN2A and may be considered a predictor of the syndrome.

Diagnosis

Thyroid ultrasonography, ultrasound-guided fine-needle aspiration cytology, and measurement of serum calcitonin levels (basal and after injection of calcitonin-stimulating reagents, e.g. pentagastrin) represent the most sensitive diagnostic tools for medullary thyroid carcinoma. *RET* genetic analysis should always be performed when the diagnosis of medullary thyroid carcinoma has been established to verify the sporadic or hereditary nature of the thyroid malignancy.

Sporadic Form

Physical examination of the neck does not offer any significant diagnostic elements. A palpable single or multi nodular goiter is usually present. A classical workup for thyroid nodular disease is then performed. Thyroid ultrasonography usually shows a hypoechoic nodule, sometimes with microcalcifications; ^{131}I and/or ^{99}Tc thyroid scintiscan reveals a cold nodule and the diagnosis is made by fine-needle aspiration cytology and/or by elevated serum calcitonin levels. Several studies have demonstrated that routine measurement of serum calcitonin is the most accurate diagnostic tool for the detection of medullary thyroid carcinoma in patients with thyroid nodules [36–42]. Subjects with elevated basal serum calcitonin should be submitted to a pentagastrin stimulation test (0.5 μg/kg intravenously) to distinguish calcitonin secreted by a medullary thyroid carcinoma: a significant increase in serum calcitonin is observed in patients with medullary thyroid carcinoma [43,44] but not in those with elevated basal serum calcitonin deriving from other sources (Table 21.2) or those due to artifacts [45–47]. Although the routine measurement of serum calcitonin in all subjects with thyroid nodules is still controversial [48,49], evidence has been provided that this approach allows an early diagnosis and treatment, thus significantly improving the outcome of this potentially lethal disease [50].

Taking into account the relevance of completeness of the first surgical treatment [51], the suspicion or clinical diagnosis of medullary thyroid carcinoma requires an accurate examination of the neck to plan the best surgical treatment for the patient. A neck ultrasound should be performed in order to identify suspicious lymph nodes with metastatic lesions to be submitted to a fine-needle aspiration for cytological examination or measurement of calcitonin in the washout of the needle used.

Since at least 5–7% of apparently sporadic medullary thyroid carcinoma are found to be hereditary cases [20,21], a preoperative evaluation of both the adrenal and parathyroid morphology and function should always be performed. The familial history should also be carefully reconsidered with particular regard to the occurrence of pheochromocytoma and hyperparathyroidism in other family members.

Measurement of the serum carcinoembryonic antigen (CEA) is also indicated in the preoperative phase because elevated levels are strongly suggestive of advanced disease. Cases with advanced local disease demonstrated by neck ultrasound and associated with elevated

Table 21.2 Hypercalcitoninemia in conditions other than MTC

"Small cell" lung carcinoma
Various neuroendocrine tumors
Chronic renal failure
Pernicious anemia
Zollinger's syndrome
Lymphocytic thyroiditis
Follicular and papillary thyroid microcarcinoma

serum CEA levels should be studied by computerized tomography (CT) to better evaluate the relationship of the disease with the large veins, trachea, and esophagus and plan the most appropriate surgical treatment [52–54].

Hereditary Forms

The hereditary nature of the tumor may be suspected on the basis of a positive family history (other members already affected), or the association with other endocrine neoplasia (pheochromocytoma and/or parathyroid adenomas) or other disorders (neuromas, marfanoid features, CLA). The evaluation of thyroid nodule in the hereditary form is performed in the same way as that recommended for sporadic cases, while the hereditary forms require mandatory simultaneous examination of adrenal and parathyroid glands.

With the exception of a few examples [55,56], the development and the diagnosis of pheochromocytoma usually follows the development and the diagnosis of medullary thyroid carcinoma. Symptoms of pheochromocytoma are not specific and may be confused with those caused by anxiety. Hypertension is very rare, especially at the beginning of the disease. An elevated value of the daily urinary excretion of epinephrine is observed as the first alteration of catecholamine production. Norepinephrine usually increases only later in the course of the disease; thus the earliest biochemical abnormality is an elevated ratio of epinephrine to norepinephrine [57,58]. It has recently been demonstrated that the measurement of plasma metanephrines, the *o*-methylated metabolites of catecholamines, offer great advantages for an early diagnosis of pheochromocytoma over standard measurements of plasmatic catecholamines. Tests for plasma metanephrines are more specific and sensitive than those for catecholamines: while normal plasma concentrations of metanephrines exclude the diagnosis of pheochromocytoma, normal plasma concentrations of catecholamines do not [59,60]. Once the biochemical suspicion of a pheochromocytoma has arisen, an abdomen ultrasound with or without computerized tomography (CT) and/or magnetic resonance imaging (MRI) may be useful for the localization of the adrenal mass [61,62]. If there is no demonstrable adrenal mass by CT or MRI scanning, ^{131}I metaiodobenzylguanidine, a catecholamine analogue actively concentrated by chromaffin tissue, can be used to investigate the presence of an extra-adrenal tumor [63].

Parathyroid glands may also be involved in MEN2A. Both adenomas and hyperplasia may be associated with an increase of the parathyroid hormone secretion, resulting in hypercalcemia and hypercalciuria in more advanced cases [64]. The earliest serum abnormality detected is a moderately elevated level of serum parathyroid hormone with normal–high levels of calcemia. In doubtful cases, a calcium infusion test that is unable to suppress the parathyroid hormone secretion will be helpful for the diagnosis [65].

RET genetic analysis is fundamental for the early discovery of gene carriers who have to be submitted to a clinical evaluation as soon as the mutation has been revealed in their constitutive DNA. Thyroid ultrasound and a serum calcitonin measurement should be performed in gene carriers. If both of them are negative a pentagastrin stimulation test is usually required. Therapeutic strategies and follow-up protocols will be adjusted according to the guidelines for the diagnosis and treatment of the multiple endocrine neoplasia as reported below [29].

Follow-up and Diagnosis of Persistent Disease

After the initial therapy has been performed, serum basal and pentagastrin-stimulated calcitonin should be measured to verify the completeness of the treatment. The first control after surgery should be done 3 months after the surgical treatment, including physical examination, neck ultrasound and measurement of serum free triiodothyronine (FT_3), free thyroxine (FT_4), thyroid-stimulating hormone (TSH), calcitonin, and CEA. Measurement of FT_3, FT_4, and TSH are requested for monitoring the levothyroxine (L-T_4) replacement therapy. Serum calcitonin and CEA measurement and neck ultrasound are necessary for follow-up of the medullary thyroid disease. Due to the prolonged half-lives, if performed too early, measurement of serum calcitonin may be misleading, especially if a high serum concentration was present preoperatively [66] (Figure 21.5). If basal calcitonin is undetectable, a pentagastrin

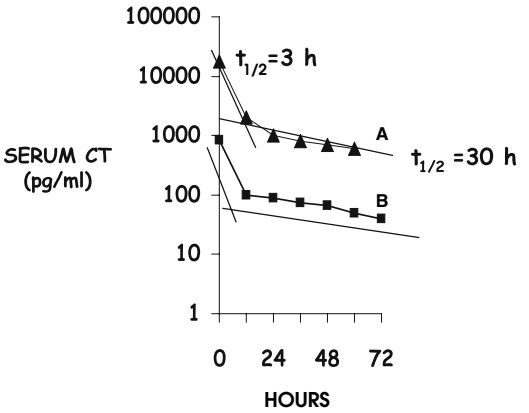

Figure 21.5 Disappearance rate of serum calcitonin (CT) after total thyroidectomy in two patients affected by MTC (A and B): two different half-lives of 3 and 30 hours respectively have been observed in both patients. (Modified from Fugazzola et al. [66].)

stimulation test is recommended. Patients with a negative pentagastrin stimulation test should be reevaluated one year later. A large series of patients with prolonged follow-up has shown that 3.3% of patients with one postoperative negative pentagastrin tests subsequently become positive [67]. Two negative pentagastrin tests on two follow-up evaluations strongly suggest that the patient is disease-free. Thus, basal serum calcitonin measurement on an annual basis is recommended, while the pentagastrin stimulation test may be performed at longer intervals (e.g. every 5 years). In patients with undetectable levels of serum calcitonin, measurement of CEA is not necessary.

Frequently basal and/or pentagastrin-stimulated serum calcitonin is persistently elevated after initial surgery. Because serum calcitonin is a very sensitive and specific tumor marker, the finding of detectable serum levels of basal or stimulated calcitonin is an indication of persistent disease. In patients with persistent disease, serum CEA concentration should be monitored because both high and increasing levels are strongly suggestive of a progressive disease [68,69]. In the majority of cases, the challenge is to find the source of production of calcitonin and CEA. An accurate neck ultrasound is the first localization technique to be performed due to the high frequency of local recurrence and cervical node metastases. A total body CT scan and bone scintigraphy are also suggested in the workup of a patient with detectable values of serum calcitonin. Other imaging techniques such as Octreoscan, ^{123}I-MIBG, and positron emission tomography (PET) may be useful although at present they do not appear to be particularly sensitive, especially in the presence of micrometastases [70–73]. The most accurate technique for the localization of occult metastases is probably the measurement of serum calcitonin after selective venous sampling catheterization: the presence of a gradient in the neck, in the mediastinum or in the suprahepatic veins suggests the presence of metastatic disease in the area where the higher levels of serum calcitonin have been found. It should be taken into account that this method is rather invasive and does not significantly improve the rate of cure [74–76].

About 50% of patients not cured at surgery have no evidence of metastatic disease when studied with the traditional imaging techniques (CT, MRI, PET). In this condition of "biochemical disease," characterized by the persistence of detectable levels of basal and/or pentagastrin-stimulated serum calcitonin but without evidence of metastatic lesions, the most widely accepted therapeutic strategy is that of "wait and see." A detectable serum calcitonin level is in fact compatible with long-term survival, during which calcitonin may remain stable with time or slowly increase. These patients are periodically monitored at intervals of 6 months to 1 year (Figure 21.6).

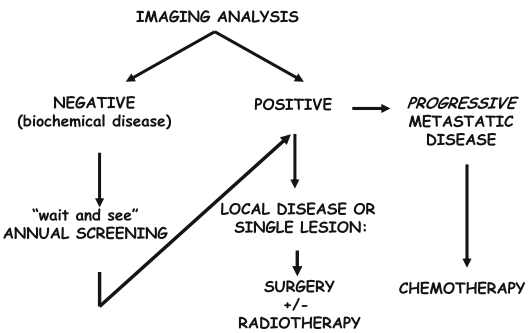

Figure 21.6 Flow chart for the management of patients with detectable serum levels of calcitonin after total thyroidectomy.

Diagnostic Tools

Fine-Needle Aspiration Cytology (FNAC)

Fine-needle aspiration is performed according to the standard procedure. In a typical cytological smear of a medullary thyroid carcinoma, cells are usually isolated, with shape varying from oval to round, large polygonal or spindled. Cytoplasm may be abundant or scanty and usually contains acidophilic granulation visible with specific stains (May–Grünwald–Giemsa). Nuclei, of which there are two or even more, are preferentially round and eccentrically localized (Figure 21.7). Amyloid is frequently detectable as clumps of amorphous material, and revealed by Congo red staining [77,78]. Immunocytochemistry for calcitonin and/or chromogranin should be performed if a diagnostic uncertainty is present [79,80]. Although the cytological pattern of medullary thyroid carcinoma is generally typical, there are several series that show a high percentage of failure in making a presurgical diagnosis [81,36–38]. Among other explanations, negative results might be due to the fact that medullary thyroid carcinoma could be present in one nodule not submitted to FNAC, especially when multinodular goiter is the clinical diagnosis. In this condition, serum calcitonin measurement is more reliable, since it is elevated even in the presence of microfoci of medullary thyroid carcinoma [36,39].

Serum Basal and Stimulated Calcitonin

Calcitonin is the most specific and sensitive medullary thyroid tumor marker, both before and after thyroidectomy [2,82,83]. It is a small polypeptide hormone of 32 amino acids normally produced almost exclusively by C cells. The gene encoding for calcitonin is located on chromosome 11p and yields two distinct messenger RNAs (mRNA) by alternative splicing: calcitonin and calcitonin gene-related peptide (CGRP) [84,85]. Calcitonin mRNA is found almost exclusively in the thyroid and CGRP mRNA in the nervous system. However, aberrant expression of CGRP may be observed in medullary thyroid carcinoma [86–88].

Release and secretion of calcitonin is mainly regulated by extracellular calcium concentration [89]. Other substances, such as pentagastrin, β-adrenergic agonists, growth hormone-releasing hormone and other gastrointestinal peptides [90–92], can stimulate calcitonin release from C cells.

The physiological role of calcitonin is still not well defined. It binds specifically to the osteoclasts and inhibits bone resorption in this site [93]. Experimental data obtained from mice

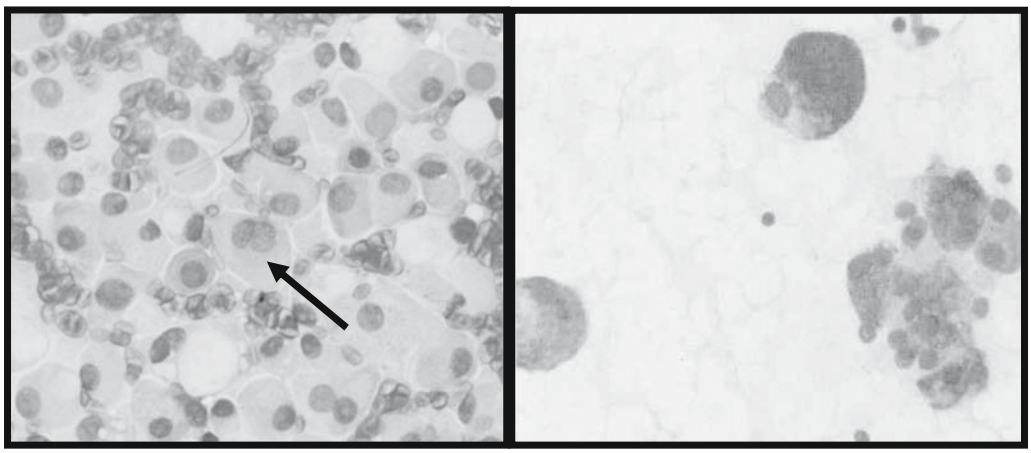

Figure 21.7 Cytological appearance of MTC. **A** Cells with abundant cytoplasm are visible; arrow indicates one cell with two nuclei, eccentrically localized (Papanicolaou staining, ×500). **B** Positive cytoplasmic staining for calcitonin confirms the suspicion of medullary carcinoma (immunocytochemistry for calcitonin, ×630). (Kindly provided by Dr G. Di Coscio, Department of Pathology, University of Pisa, Italy.)

homozygous null for the calcitonin gene have demonstrated a significant increase in bone formation at 1 and 3 months of age [94]. However, in normal adult human subjects even quite large doses of calcitonin have little effect on serum calcium levels. It is only in subjects with an increased bone turnover that calcitonin treatment acutely inhibits bone resorption and lowers the serum calcium [95]. Recently, evidence has been reported suggesting that the actions of calcitonin may not be limited to bone. Calcitonin receptors have also been identified in the central nervous system, testes, skeletal muscle, lymphocytes, and the placenta [96].

Ten years after the recognition of medullary thyroid carcinoma as a distinct histological type of thyroid carcinoma [1], high levels of calcitonin were demonstrated to be present both in the tumoral tissue and serum of patients with medullary thyroid carcinoma [2]. Elevated basal levels of serum calcitonin are diagnostic of medullary thyroid carcinoma. However, there are several other conditions, both physiological and pathological, in which basal levels of serum calcitonin may be found to be elevated and a differential diagnosis may be indicated [45,97–101]. Since the release of calcitonin in these diseases does not appear to be regulated by the same factors that stimulate calcitonin release in the C cells, differential diagnosis can be performed by either the calcium (2 mg/kg) or pentagastrin (0.5 mg/kg intravenously) rapid stimulation test [102]. While in patients with medullary thyroid carcinoma and elevated basal levels of calcitonin, the pentagastrin stimulation determines a 5–10-fold increase in serum levels of calcitonin, in other diseases the calcitonin increase is limited or absent. In patients with an endocrine tumor of another origin, an increase may be observed but is not usually greater than twofold [43,102].

Routine measurement of serum calcitonin in nodular thyroid diseases allows the preoperative diagnosis of unsuspected sporadic medullary thyroid carcinoma [36–42]. Calcitonin screening determines the early diagnosis of medullary thyroid carcinoma, usually when the tumor is still at stage I, thus favoring successful surgical treatment. A comparison of the outcome of two groups of patients, one diagnosed by serum calcitonin screening and the other by cytology or histology, has demonstrated a significantly better prognosis in the first group [50] (Figure 21.8).

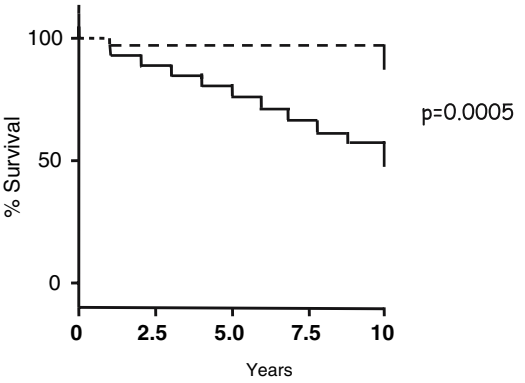

Figure 21.8 Significant difference in survival rate between patients with MTC diagnosed by serum calcitonin screening (dashed line) and those with MTC diagnosed at surgery and/or by preoperative cytology (solid line). (Modified from Elisei at al. [50].)

It is worth noting that calcitonin precursors (pre- and pro-calcitonin) and post-translational deriving peptides (katacalcin and N-terminal peptide) are also present in the blood and may interfere in the measurement of serum calcitonin. Artifactual recognition of larger calcitonin precursors is commonly observed with one-site radioimmunoassay. This problem seems to be overcome by the most recent generation of calcitonin two-site immunoradiometric assays (IRMA) that are able to specifically recognize the mature molecule of calcitonin [103]. Artifacts may be also determined by the presence of heterophilic antibodies in the blood of patients, which can interfere with the assay, thus producing false-positive results [47]. The absence of a significant increase in the serum calcitonin levels after pentagastrin or calcium stimulation test strongly suggests the artifactual nature of these false-positive values.

As an additional tool for the diagnosis of medullary thyroid carcinoma, calcitonin measurement in the washout of the needle used for the puncture of a suspected thyroid nodule may be useful. This approach is of particular diagnostic utility to ascertain the nature of neck lymph nodes, especially before thyroidectomy, to plan the surgical approach or the most appropriate therapeutic strategies.

Other Secretory Products

Although calcitonin is the most reliable tumor marker due to its high sensitivity and specificity, there are some other proteins that may be released by the malignant transformed C cell.

Serum CEA is usually elevated when the disease is diffuse and distant metastases are present [68,69]. Unlike calcitonin, CEA does not show any response to the pentagastrin stimulus. It is most useful in monitoring the progression of the disease since its level increases when the disease becomes rapidly progressive.

Serum chromogranin may also be elevated in patients with medullary thyroid carcinoma. It is not specific since elevated values have been reported in patients with neither clinical nor biochemical evidence of a primary medullary thyroid carcinoma [104].

As in many other neuroendocrine tumors, somatostatin, gastrin-releasing peptide, vasoactive intestinal peptide, neuron-specific enolase, and other neuroendocrine substances may be produced abnormally but none of these peptides are useful for diagnosis [105–107].

Some of the products of medullary thyroid carcinoma may result in significant clinical manifestations: not just CGRP, but also vasoactive intestinal peptide, serotonin and prostaglandins, may all contribute to the flushing and diarrhea syndrome [108,109].

Histology

Normal C Cells

The parafollicular C cells represent about 1% of all thyroid cells and are located at the basal layer of the follicle (Figure 21.9). At variance with thyroid follicular cells, which derive from endoderm, C cells originate from the neural crest and migrate to the final location along with the ultimobranchial body, during embryonic development [110,111]. Although C cells have several features that differentiate them from follicular epithelium, there is evidence to suggest the possible origin of the follicular and parafollicular C cells from a common ancestral cell. Critical neurotrophic growth factors, including glial-derived neurotrophic factor (GDNF), which is a natural ligand of RET receptor, as well as nerve growth factor (NGF) and other neurotrophins, seem to play a central role in promoting the differentiation of cells deriving from the neural crest [112].

There are specific features that make the C cell a separate entity from a follicular cell: (a) the peculiar distribution in the thyroid gland,

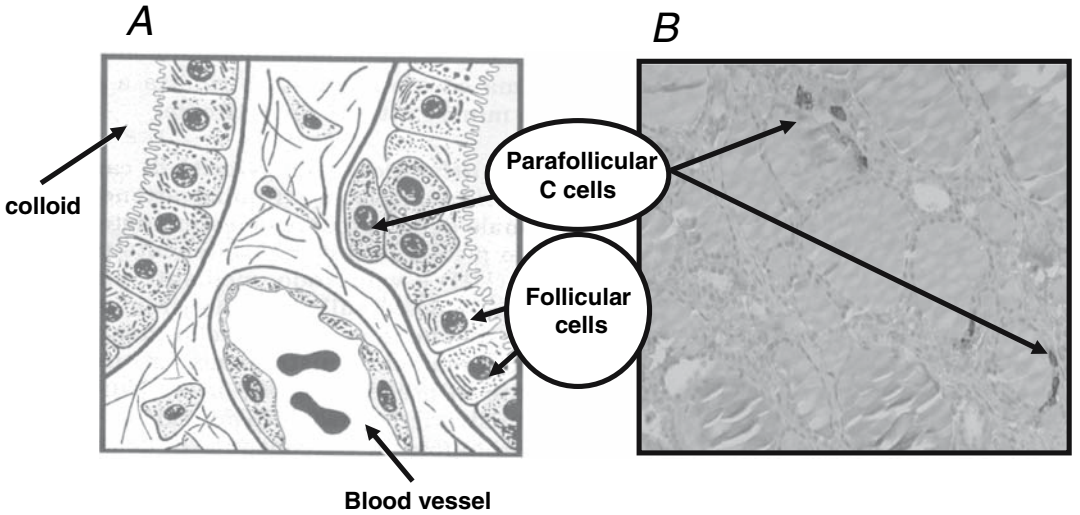

Figure 21.9 Schematic representation (**A**) and immunohistochemistry for calcitonin showing normal parafollicular C cells in normal thyroid tissue (**B**, ×100): the parafollicular C cells are located at the basal layer of the follicle.

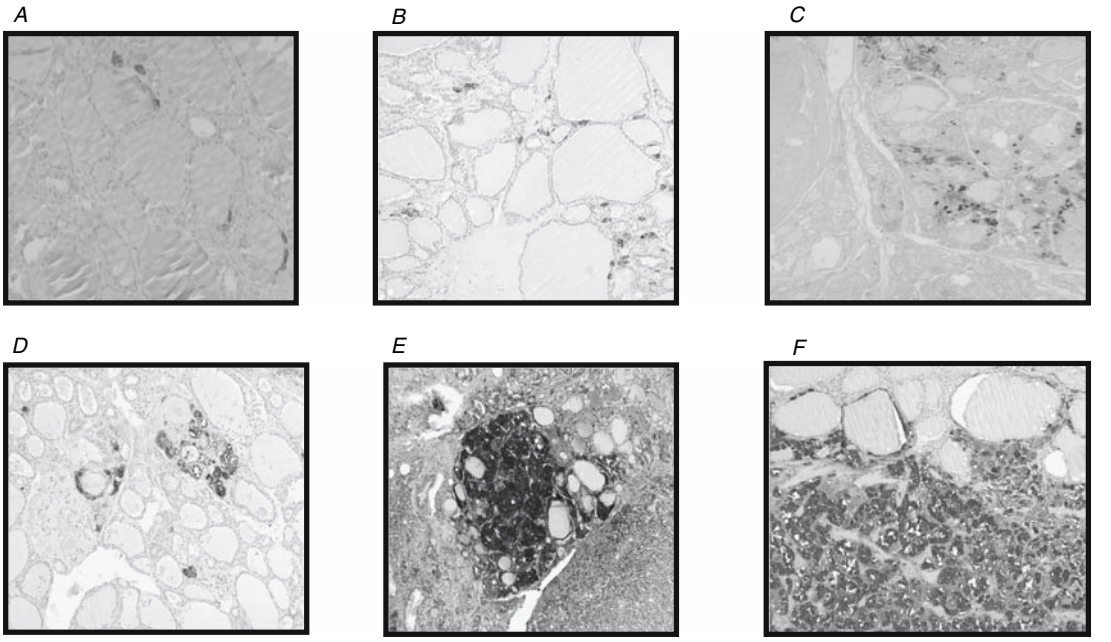

Figure 21.10 Different stages in the development of hereditary MTC. **A** Normal parafollicular C cells; **B** slight C-cell hyperplasia (CCH); **C** diffuse CCH; **D** focal CCH; **E** nodular CCH; **F** MTC. Immunohistochemistry for calcitonin (Ventana Medical System antibody, 1 : 100; ×100). (Kindly provided by Professor F. Basolo, Department of Pathology, University of Pisa, Italy.)

which is prevalent at the junction of the upper third and the lower two thirds and along the central vertical axis of each thyroid lobe; (b) the growth and functional independence from TSH, as well as the inability to take up iodine; and (c) the production and secretion of calcitonin, a biogenic amine, which is almost exclusively produced by both normal and malignant C cells.

C-Cell Hyperplasia

The definition of C-cell hyperplasia has changed over the years, especially after the introduction of *RET* genetic screening and the histological examination of apparently normal thyroid glands of mutated gene carriers that usually show an increased number of C cells. Studies of both human normal thyroid and thyroids affected by lymphocytic thyroiditis have demonstrated that one can see up to 50 C cells per 1 and 3 low power fields respectively, without correlation with any pathological status [98,113]. According to these findings, this is at present the most widely accepted definition of C-cell hyperplasia, even though this criterion may be not respected in the presence of cytologically evident atypia [114].

According to the number and the distribution of C cells either a diffuse, focal, or nodular C-cell hyperplasia can be distinguished (Figure 21.10). It is likely that they represent progressive stages through which the normal C cell is transformed into a tumoral cell. While there is general agreement in considering C-cell hyperplasia the preneoplastic lesion of the hereditary form of medullary thyroid carcinoma, little is known about the relationship between C-cell hyperplasia and the sporadic form. Nevertheless, about 30% of sporadic medullary thyroid carcinoma is associated with C-cell hyperplasia [115].

Several authors would like to distinguish two types of C-cell hyperplasia: primary or neoplastic C-cell hyperplasia, which is related to the hereditary form of medullary thyroid carcinoma, and secondary or non-neoplastic C-cell hyperplasia, which may be observed in other thyroid diseases (thyroiditis and follicular or papillary microcarcinoma) and in about 20% of normal subjects [116,117]. However, the

pathological definition and clinical significance of secondary C-cell hyperplasia remains unclear.

Medullary Thyroid Carcinoma

Under macroscopic examination, medullary thyroid carcinoma shows a hard and firm consistency and is either chalky white or red in color on cross-section. Histologically, medullary thyroid carcinoma is pleomorphic with spindle-shaped or rounded cells characteristically organized in a nested pattern. Mitoses are not very frequent, nuclei are usually uniform, and the eosinophilic cytoplasm is characterized by the presence of secretory granules. Deposits of amyloid substance are frequently (60–80%) observed between tumoral cells [118].

Sometimes there is difficulty distinguishing medullary thyroid carcinoma from anaplastic carcinoma, Hürthle cell carcinoma or insular carcinoma, especially if pseudopapillary elements or giants cells are present. Positive immunohistochemistry for calcitonin is diagnostic of medullary thyroid carcinoma. Immunohistochemistry for chromogranin A and carcinoembryonic antigen may also be useful [115] (Figure 21.11).

Histopathological description of medullary thyroid carcinoma must include the number and the distribution of tumoral foci as well as the simultaneous presence of C-cell hyperplasia. This information is of practical usefulness because bilaterality, multicentricity, and C-cell hyperplasia are considered the histological hallmarks of the hereditary forms [22].

A mixed form of medullary thyroid carcinoma is also described [119]. It is characterized by the simultaneous presence of parafollicular and follicular cell features, with positive immunohistochemistry for both calcitonin and thyroglobulin. In this regard, it is worth noting that the association of medullary and papillary thyroid carcinoma in the same thyroid gland seems to be quite frequent [120,121]. Molecular studies have shown that genes theoretically specific for the parafollicular C cells (i.e. normal *RET* gene) are expressed in papillary thyroid carcinoma and that genes theoretically specific for follicular cells (e.g. thyroglobulin, TSH receptor, thyroid transcription factor 1) are expressed in medullary thyroid carcinoma [122–124]. Despite all these observations, it is still controversial whether the mixed medullary thyroid carcinoma is a real separate histological entity, originating from an ancestral stem cell able to differentiate as both follicular and parafollicular cell, or the consequence of the collision of two distinct tumors, medullary and papillary, originating in the same thyroid gland.

RET Genetic Analysis

RET mutation analysis represents one of the most useful genetic screening tests in clinical practice. The mutation is inherited as an autosomal dominant trait: since the penetrance of *RET* mutations is near 100%, all gene heterozygous carriers will develop medullary thyroid

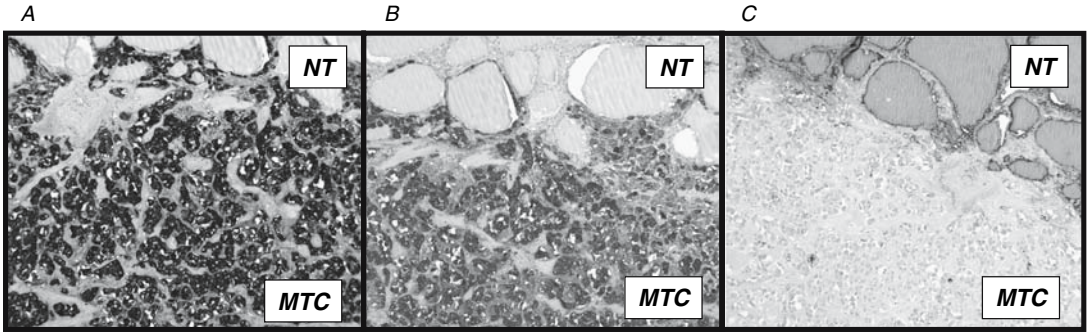

Figure 21.11 Medullary thyroid carcinoma (MTC) and normal adjacent thyroid tissue (NT). **A** Immunohistochemistry for calcitonin. **B** Immunohistochemistry for chromogranin. **C** Immunohistochemistry for thyroglobulin. Both calcitonin and chromogranin, but not thyroglobulin, are positive in MTC. A positive immunostaining for thyroglobulin is present in NT (×100, Ventana Medical System antibodies). (Kindly provided by Professor F. Basolo, Department of Pathology, University of Pisa, Italy.)

carcinoma, which is lethal in almost 50% of cases if not adequately treated. The genotype–phenotype correlation has been well demonstrated by the analysis of 477 MEN2 families studied by the International RET Consortium: no evidence of false-positive *RET* mutation was described and all patients who underwent thyroidectomy on the basis of the genetic screening were found to have medullary thyroid cancer [27]. Recently, a new mutation at codon 883 in exon 15 has been reported to result in the development of medullary thyroid carcinoma only in the homozygous condition [125].

Screening for *RET* gene mutations allows the early discovery of gene carriers, who can be treated with precocious and even prophylactic thyroidectomy, which may provide a definitive cure of this potentially lethal thyroid disease [126].

RET Gene

The *RET* proto-oncogene is a 21 exon gene that lies on chromosome 10q11-2 and encodes for a tyrosine kinase transmembrane receptor. The receptor is composed of an extracellular domain (EC), with a distal cadherin-like region and a juxta-membrane cystine-rich region, a transmembrane domain and an intracellular domain with tryosine kinase activity (TK) (Figure 21.12). *RET* is expressed in a variety of neuronal cell lineages including thyroid C cells and adrenal medulla. Recently data indicate that

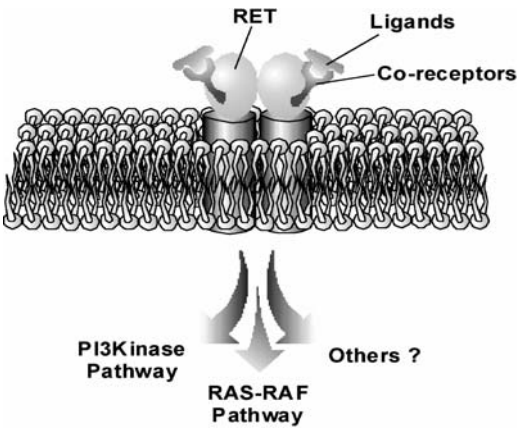

Figure 21.12 Schematic representation of RET tyrosine kinase receptor. The interaction with the ligand and corresponding co-receptor induces the dimerization and phosphorylation of the receptor, resulting in the activation of the intracellular signaling pathway.

RET gene expression may also occur in follicular thyroid cells [122]. In physiological conditions, the activation of RET protein is secondary to its dimerization due to the interaction with one of its ligands. Four different ligands have so far been recognized: GDNF, neurturin (NTN), persepin (PNS), and artemin (ART). The interaction is mediated by a ligand specific co-receptor (e.g. the GFRα-1 is the co-receptor for the GDNF). The dimerization of RET protein induces autophosphorylation of the TK domain and the activation of downstream signaling pathways [127].

In 1987 genetic linkage analysis localized MEN2 to the centromeric region of chromosome 10 [128,129]. In 1993 two independent groups reported that activating germline point mutations of the *RET* proto-oncogene are causative events in MEN2A and in FMTC [8,9] (Figure 21.13). One year later, MEN2B was also associated with germline *RET* proto-oncogene mutations [130]. Since then, a large number of publications have addressed the relationship between *RET* mutations and the clinical phenotype of MEN2 patients and the clinical implication of screening MEN2 family members for *RET* gene mutations.

About 98% of MEN2A cases are associated with *RET* mutations in the cystine-rich extracellular domain, in particular in codons 609, 611, 618, 620, and 634 of exons 10 and 11. Mutations at codon 634 of exon 11 (mainly TGC to CGC) are the most common, accounting for 85% of MEN2A cases [27–29,131]. Interestingly, mutation of cystine 634 significantly correlates with the presence of pheochromocytoma and parathyroid adenomas (Table 21.3).

A specific mutation in exon 16, at codon 918 (ATG to ACG) is almost invariably associated with MEN2B. The substitution of methionine with threonine causes alterations in the substrate recognition pocket of the catalytic probe determining the activation of the intra-signaling pathways. Other rare mutations of the intracellular domain have been reported in codon 883 of exon 15 [132]. A double *RET* mutation at codon 804 and 904 has also been described [133]. The Met918Thr mutation is associated with a very aggressive MTC that usually develops during childhood, often only a few years after birth.

In FMTC, the mutations are widely distributed among the five cystine codons 609, 611, 618, 620, and 634 but also in other non-cystine codons, such as codon 804 in exon 14, 891 in

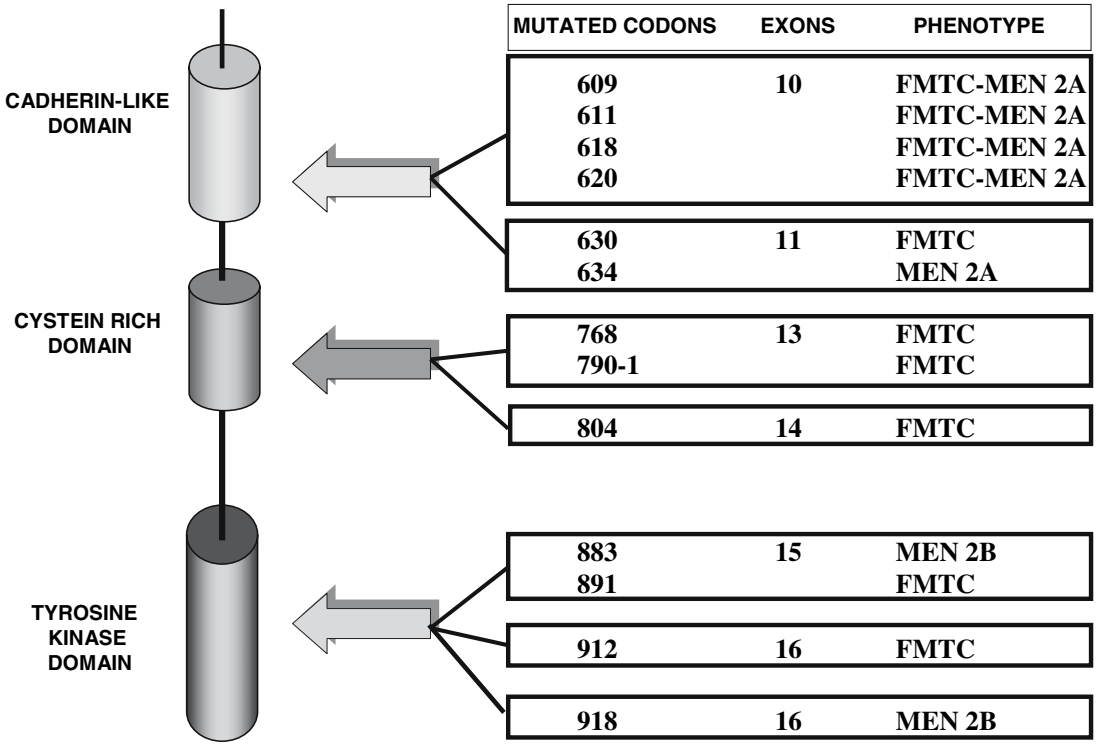

Figure 21.13 Schematic representation of *RET* gene with the location of all known mutated codons in the three main regions of the gene and the relationship with the MEN syndromes.

exon 15 and others (Figure 21.14). A different biological behavior, characterized by a lower aggressiveness and an older mean age at diagnosis, has been described for FMTC associated with mutations in non cystine codons with respect to both MEN2A and FMTC with mutations in cystine codons [28].

In about 4–10% of MEN2A or FMTC patients and in about 95% of those with MEN2B, the germline *RET* mutation is a "de novo" mutation,

Table 21.3 Correlation between phenotype and *RET* gene mutations

	Most frequently involved codons[a]							
	609	611	618	620	634	768	804	918
MEN2A								
MEN2A (1) (MTC + pheochromocytoma + hyperparathyroidism)			6%	2%	92%			
MEN2A (2) (MTC + pheochromocytoma)		3%	4%	13%	80%			
MEN2A (3) (MTC + hyperparathyroidism)	8%	15%	8%		69%			
MEN2B								97%
FMTC	7%	3%	33%	17%	30%		3%	
Sporadic FMTC								95%

[a] As a percentage of patients with somatic *RET* gene mutations.
[b] Somatic mutations detectable in about 40% of sporadic MTC.

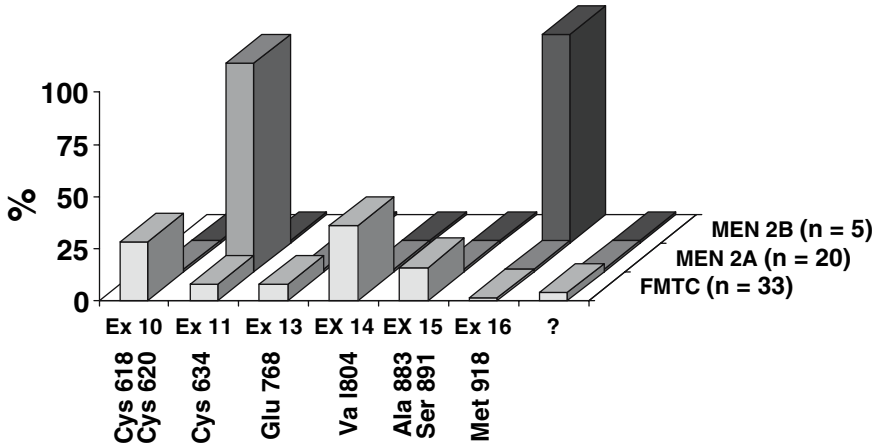

Figure 21.14 Different MEN2 syndromes and corresponding germline *RET* mutations in an Italian series of MEN2 kindreds ($n = 58$). (Series of the Department of Endocrinology, University of Pisa, Italy.)

as demonstrated by the negative finding of the *RET* genetic analysis in the patients' parents. In these cases the mutation is usually located in the allele inherited from the patient's father [134].

Somatic *RET* mutations are found in about 40% of sporadic cases of MTC mainly consisting of a Met918Thr mutation in exon 16, which is the same mutation also occurring in MEN2B (Figure 21.15). Other *RET* somatic mutations and also some small deletions have been reported in other codons [135]. Several studies indicate that MTC patients with somatic *RET* mutations have a poorer prognosis than those with no evidence of *RET* mutation [136].

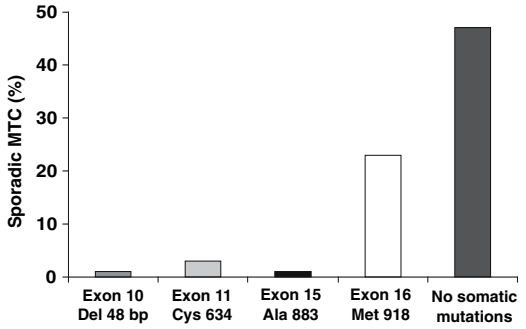

Figure 21.15 Somatic *RET* gene mutations in an Italian series of 77 sporadic MTC. About 50% of cases do not harbor any known *RET* mutation. The most frequent somatic mutation is the Met918Thr substitution at exon 16. (Series of the Department of Endocrinology, University of Pisa, Italy.)

Several *RET* gene polymorphisms have been found both in MTC affected patients and in normal subjects. It is still controversial whether some of these polymorphisms have a higher prevalence in MTC with respect to normal individuals and if they play any role in the development of MTC [137–139].

Screening for *RET* Gene Mutations in MEN2 Family Members

The recognition of the role of RET mutation in MEN2 provided a reliable method to screen family members of an affected proband carrying a germline mutation. From a practical point of view, once the germline *RET* mutation of the index case has been recognized, blood is taken from all first degree family members. Informed consent and adequate genetic counseling are requested. This allows the identification of "gene carriers" at the time they are still clinically unaffected or at an early stage of the disease. It also has the advantage of excluding "non gene carriers" from further testing for the rest of their life. Although the presence of a germline *RET* mutation is diagnostic of MEN2 syndrome, gene carriers must be submitted to further clinical and biochemical evaluations to ascertain the actual development of the MTC and its extension, if already present. The involvement of other endocrine organs must also be assessed [29,140].

Screening for *RET* Gene Mutations in Apparently Sporadic Cases

All patients with MTC, independently from their apparent sporadic origin, should be submitted to genetic screening of the *RET* gene by analyzing their constitutional DNA derived from blood and, whenever possible, also from tumoral tissue. It is well known that from 5% to 10% of apparently sporadic MTC cases are found to harbor a germline *RET* mutation being "de novo" or misdiagnosed familial cases. This finding is of great relevance for the early discovery of the other gene carriers in the family who are unaware of their condition.

In sporadic MTC cases, *RET* gene analysis should be in any case performed also in the tumoral tissue, collected at surgery and kept at –80°C or in the paraffin-embedded tumoral tissue. There are at least three main reasons to justify this procedure: (a) the discovery of a somatic mutation, which usually occurs in 45% of cases, strongly supports the sporadic nature of the tumor; (b) the prognostic value of the presence/absence of the somatic mutation; (c) the future possibility for *RET* mutated patients to be treated with drugs specifically aimed at inhibiting the altered *RET* gene.

Therapy

Initial Treatment

An early diagnosis and complete surgical treatment are the bases for a definitive cure of patients affected by medullary thyroid carcinoma (Figure 21.16). The minimal standard procedure is total (or near-total) thyroidectomy with central neck lymph node dissection, in both sporadic and familial forms. The need for total thyroidectomy is supported by the multicentricity and bilaterality of the medullary thyroid carcinoma that occurs in about 100% of the hereditary form and 30% of sporadic form [22]. Furthermore, C-cell hyperplasia, which is considered a preneoplastic lesion, is almost invariably associated with the hereditary form of medullary thyroid carcinoma and, to a lesser extent, with the sporadic form [24]. An additional reason in favor of total thyroidectomy is the fact that, as mentioned above, 5–7% of apparently sporadic cases are in fact hereditary forms, which almost invariably have bilateral disease [20,21].

Central node dissection, from the hyoid bone to the innominate veins, is mandatory during the initial operation. This node compartment is in fact the primary lymphatic drainage of the thyroid and 50–60% of medullary thyroid carcinomas show node metastases in this area at the time of presentation [51,141]. The removal of the central compartment also has a prophylactic significance and must be performed independently of the size of the primary tumor and the presurgical evidence of lymph node involvement. This surgical approach is also suggested in *RET* gene carriers without clinical evidence of the disease, which can be completely cured by surgical treatment. It is still controversial as to whether the central node dissection has to be performed in child gene carriers without clinical and biochemical (undetectable levels of basal and stimulated calcitonin) evidence of disease [142–144].

Whenever a presurgical clinical diagnosis of node metastases is achieved, the surgical dissection of the corresponding lateral node compartment should be included. It is still debated whether a modified radical neck dissection with removal of nodes in the ipsilateral or bilateral compartment should be performed in any case. Since unilateral or bilateral cervical nodal metastases occur in up to 90% of patients with medullary thyroid carcinoma, especially when

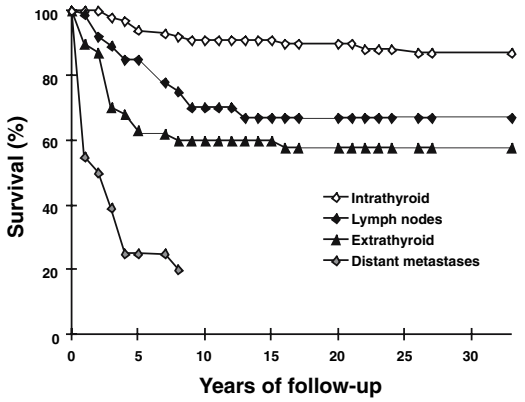

Figure 21.16 Different survival rates of patients affected by MTC according to the tumor extension at the time of the diagnosis: The best prognosis is observed in patients with MTC at stage I. (Modified from Gharib et al. [15].)

the primary tumor is palpable [39,55], several authors strongly suggest an "en bloc" dissection of both central and bilateral neck compartments together with the thyroid gland [145]. This is of great clinical significance because the adequacy of the initial surgical treatment is a prerequisite for the effective cure of the MTC, thus the choice of the most appropriate initial procedure is fundamental. In this regard it is worth considering that radical neck dissection may result in significant morbidity and has not been clearly shown to improve the prognosis of the disease, which in fact is dependent on several other factors such as the local extent of the disease at the time of diagnosis, the presence or absence of other endocrine neoplasia, and, the presence of cervical lymph node metastases. Patients who are cured by the initial treatment are usually those with a lower stage of disease, with no involvement of lateral nodes in the neck. Several reports indicate that when lateral node metastases are present at diagnosis, achievement of a definitive cure with the initial treatment is uncommon regardless of the extent of the surgical procedure [67,146,147].

C cells are not able to actively concentrate radioiodine and, as consequence, ^{131}I radioactive ablation is not useful in medullary thyroid carcinoma. There are only a few anecdotal reports indicating a beneficial effect of ^{131}I treatment of the postsurgical remnant presumably due to death of C cells adjacent to follicular cells as consequence of a bystander effect [148,149].

In patients with a locally aggressive disease not completely removed by the primary resection, surgical treatment should be followed by external-beam radiotherapy as adjuvant treatment. Although bulky medullary thyroid carcinoma deposits are consistently resistant to external radiotherapy, there is evidence of a potential benefit from radiotherapy in terms of a lower risk (from two- to fourfold) of local recurrence in patients with residual disease [150,151]. This procedure should be reserved for patients who have undergone complete central compartment and lateral neck dissection and postponed to a second surgical treatment in those who have been approached with a less aggressive primary resection. Radiation therapy after thyroidectomy and node dissection is not generally recommended on a prophylactic basis but it is worth mentioning that there is some evidence that external beam radiation in patients with residual malignant disease after surgery may increase the 5-year survival rate from 60% to 95% [152].

Hormone replacement therapy with L-thyroxine (L-T_4) should be started immediately after thyroidectomy. Unlike papillary and follicular thyroid tumors, medullary carcinoma is not dependent on TSH for both growth and function, thus there is no need to treat patients with L-T_4 suppressive therapy: the daily dose should be tailored by measuring serum FT_3, FT_4, and TSH aiming to keep their values within the normal range. Unilateral or bilateral adrenalectomy must be performed before total thyroidectomy, when a pheochromocytoma has been documented, because of the risk of a life-threatening hypertensive crisis during the induction of anesthesia for the neck surgical treatment. Preoperative screening for pheochromocytoma should be conducted in all patients with a diagnosis of medullary thyroid carcinoma since the patient may be an index case of a familial form, presented as apparently sporadic. Although pheochromocytoma is usually bilateral, a 10-year interval is the mean period between the first and the contralateral adrenal mass appearance. Different approaches to the management of adrenal medullary disease are suggested when only one adrenal gland is involved at the time of the diagnosis. Bilateral adrenalectomy in principle eliminates the need for a second intervention later in the patient's life, but implies the risk associated with the corticosteroid deficiency. Since a laparoscopic surgical approach has been introduced [153], the preferred strategy is to remove only the affected adrenal gland and periodically monitor the morphology and function of the other adrenal gland. Whatever the final decision, all patients to be submitted to adrenalectomy should be treated preoperatively with pharmacological α- and β-adrenergic antagonists [154,155].

Grossly enlarged parathyroid glands should be resected during the first operation for patients with hereditary forms of medullary thyroid carcinoma and documented clinical hyperparathyroidism. An intraoperative serum parathyroid hormone measurement is recommended to ensure the precise and total removal of the affected gland(s). This procedure is of practical importance especially when the macroscopic appearance of the removed parathyroid is not indicative of the presence of adenoma, suggesting the presence of multiple adenomatosis or diffuse hyperplasia [156]. In

some centers normal or hyperplastic parathyroid glands of patients with hereditary forms are always removed, even in the presence of normal serum parathyroid hormone levels. They are appropriately marked, for making their localization easier whenever it might be necessary, and totally or partially implanted in a muscle [157]. It is worth noting that an aggressive management of normal parathyroid glands is associated with a higher incidence of hypoparathyroidism. In this regard, a greater concern is represented by young *RET* gene carriers who, if rendered hypoparathyroid, would be exposed to the need for calcium and vitamin D supplementation for the rest of their lives.

Gene Carrier Treatment

Once a gene carrier has been diagnosed by genetic analysis, the therapeutic strategy should be defined according to the guidelines for the diagnosis and treatment of multiple endocrine neoplasia [29], which take into account the different biological behavior of the medullary thyroid carcinoma in the three forms of multiple endocrine neoplasia. In MEN2B total thyroidectomy should be performed as soon as possible, even under 2 years of age if the diagnosis is available. In MEN2A, total thyroidectomy should be performed at 10 years of age or less if the pentagastrin stimulation test for calcitonin is positive. In FMTC, yearly based follow-up should be performed with a pentagastrin stimulation test and thyroid surgical treatment is indicated at the first positive test.

Parathyroid and adrenal gland morphology and function must be assessed and an adequate treatment should be performed if needed. If no abnormalities of these glands are found at the time of diagnosis, their morphology and function should be monitored annually because both hyperparathyroidism and pheochromocytoma may show up later in life.

Further Treatments

When patients are not cured by the primary surgical treatment, other therapeutic procedures are indicated according to the localization and the number of lesions. In planning a therapeutic strategy it should be taken into account that most distant metastases found during follow-up are small at the time of their recognition and that their growth is usually very slow. These lesions are compatible with a long period of good quality of life. In these cases, an aggressive therapeutic approach may not be indicated, unless an evident rapidly progressive disease is demonstrated.

Local Recurrence and Regional Lymph Node Metastases

In the first years following surgical treatment the regional lymph nodes of the neck and mediastinum are most frequently responsible for persistent disease. A second surgical treatment with a curative intent is recommended only for minimal residual disease. To this purpose an extensive modified neck dissection involving microdissection of all node-bearing compartments from the clavicle to the skull is recommended [158]. Unfortunately, less than 30% of patients affected by MTC with extrathyroidal invasion can be cured by a second surgical treatment [159,160]. Capsular invasion and more than 10 lymph node metastases [145] in the primary surgical specimens are significant predictors of poor response to reoperation. In the clinical management of patients with MTC the identification of those who might benefit from this treatment is of great practical importance to avoid false expectation.

If a reasonable prospect of a definitive cure is not foreseen, a second surgical treatment should only be performed for symptomatic lesions or when their growth may cause significant morbidity as may happen for lymph nodes of the mediastinum adjacent to the great vessels, tracheoesophageal groove, carotid sheath, and brachial plexus. A second operation with palliative intent may be strongly indicated in patients with compressive symptoms who can benefit from a surgical debulking. Local external radiotherapy may be indicated in these advanced situations.

Distant Metastases

Surgical treatment of distant metastases is not indicated, except for those lesions whose growth may compromise some vital functions. Surgical debulking of vertebral metastases that could impair spinal cord function is an example of a non-curative but appropriate procedure.

Chemotherapy for advanced, metastatic medullary thyroid carcinoma has shown limited response rates in several small-scale trials published to date [161]. Thus, chemotherapy should only be used in patients with a diffuse and well-documented progressive disease. In these cases the reduction in the growth rate and the stabilization of the disease represent a satisfactory result. A high dose of doxorubicin (75 mg/m^2 every 3–4 weeks) is the most effective chemotherapeutic agent with a response rate of 15–20% in terms of stabilization of the disease. The same response rate is obtained when doxorubicin is used alone or in combination with other drugs such as 5-fluorouracil, dacarbazine, streptozocin, cyclophosphamide, and vincristine [162,163]. Since major toxic effects are frequently observed and the response is only partial and short-lived, chemotherapy should not be used in patients with stable or slowly progressive disease.

Medullary thyroid carcinoma is a neuroendocrine tumor and 30–50% of cases express somatostatin receptors as ascertained by Octreoscan [70]. Over the years, different types of octreotide, from the native to the long-acting analogues, have been explored as potential therapeutic agents. In the majority of cases, a significant reduction in serum calcitonin has been demonstrated [164]. Unfortunately, no evidence of a parallel reduction in the number and/or the size of tumor lesions has been shown. Inconstant and transient effects in reducing symptoms such as flushing and diarrhea are not sufficient to recommend the administration of somatostatin analogues in metastatic medullary thyroid cancer patients. No improvement in the therapeutic effect has been observed when the somatostatin analogues have been combined with α-interferon [164]. Recently, specific somatostatin receptors have been identified both in cell lines deriving from human medullary thyroid carcinoma and in tissue surgical specimens of medullary thyroid carcinoma [165–167]. The possibility of using analogues that specifically recognize these receptors is currently under evaluation.

Treatment with several radioactive elements has been widely explored. There is no clinical evidence to encourage treatment with ^{131}I, which has been demonstrated to be ineffective in large series of patients with metastatic medullary thyroid carcinoma [148,149]. A more promising use of radioactive iodine has been shown when iodine is linked to metaiodobenzylguanidine [168,169]. However, only a small proportion of patients (30%) have a positive result and the treatment is relatively ineffective. Other radioimmunotherapeutic agents have been explored and, in particular, the bi-specific antibodies directed against the carcinoembryonic antigen (CEA), which is expressed on the surface of the majority of metastatic medullary thyroid cells, but unfortunately no significant benefits have been found in treated patients [170]. Preliminary data have been reported on the use of somatostatin analogues labeled with yttrium-90 or other radionuclides, in patients with metastatic medullary thyroid carcinoma showing octreotide uptake at Octreoscan [169,171]. Further studies are needed to establish the therapeutic effectiveness of this procedure.

As reported above, external beam radiotherapy is indicated in medullary thyroid carcinoma when local aggressive unresectable disease is present. Although the radiosensitivity of medullary thyroid carcinoma is moderate and no survival benefit has been so far demonstrated, available data suggest that improved local control of the disease with a longer interval between treatment and the recurrence of regional or local disease may be obtained by external postsurgical radiotherapy compared with those not treated [151,152]. When bone metastases are present, external beam radiation is indicated to prevent pathological fractures and for palliation of symptoms. Brain metastases also may be treated with external radiotherapy and a rapid and reliable response may be obtained. Other therapeutic strategies are indicated for lung and liver metastases. Radiation therapy of lung metastases carries risk of radiation fibrosis and respiratory dysfunction. Chemotherapy is better indicated if progression of pulmonary disease is observed. Liver metastases may be amenable to surgical resection, especially if preceded by transarterial chemoembolization. This procedure has been demonstrated to be of particular benefit when liver metastases are smaller than 3 cm and the liver involvement is less than 30% [172].

Discomfortable Syndrome

Particular care should be taken to reduce severe symptoms such as diarrhea, flushes, and

pain. Diarrhea is of particular discomfort for patients, deeply disturbing their quality of life and resulting in severe loss of weight. Symptomatic therapy should be performed using loperamide. Histamine receptor inhibitors may be employed to control the flushing syndrome. Analgesic drugs, as appropriate, should be used to give some pain relief to patients with very advanced metastatic disease.

Future Therapeutic Strategies

At present, the treatment of advanced and metastatic medullary thyroid carcinoma is unsatisfactory. Novel alternative therapeutic approaches are under investigation and several experimental studies are already ongoing [173]. Tissue-specific cancer gene therapy has been evaluated for several years. Adenovirus-mediated tumor-specific combined gene therapy using the herpes simplex virus thymidine/ganciclovir system and murine interleukin-12 seems very promising. An effective growth suppression of tumor has been observed in rat models affected by medullary thyroid carcinoma and treated with this system [174]. Other interesting approaches are based on immunotherapy, for example stimulation of immune response and vaccination with tumor lysate [175,176].

Inhibitors of tyrosine kinase receptors are currently under investigation for their potential role as therapeutic agents in human cancers related to a structural alteration of these receptors [177]. As a general mechanism, they compete with adenosine triphosphate, thus hampering autophosphorylation and signal transduction downstream from the targeted kinase receptor. At present, two drugs have been shown to produce beneficial effects: STI571 (imatinib or Gleevec), which has been documented to inhibit BCR-ABL in chronic myeloid leukemia and c-KIT and PDGFR in gastrointestinal stromal tumors, and ZD1839 (gefitinib or Iressa), which has been documented to inhibit EGFR in non-small cell lung carcinoma [178,179]. A further very promising agent is ZD6474, which has been demonstrated to have an inhibiting activity on the growth of cell lines harboring *RET* gene activating alterations and on the formation of tumors after injection of these cells into nude mice [180–182]. ZD6474 also exerts an inhibiting effect on the vascular endothelial growth factor receptor (VEGFR), resulting in antiangiogenic activity. At present, a phase I trial has been completed with no significant adverse events. Clinical trials of phase II and III are required to establish the therapeutic potential of this and other compounds in *RET*-positive cancer patients.

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