REVIEW

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Protean cytological, histological and immunohistochemical appearances of medullary thyroid carcinoma: current updates

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Abstract

Medullary thyroid carcinoma (MTC) accounts for only 0.5–3% of all malignant diseases, but is responsible for more deaths every year than all the other endocrine malignancies taken together. Approximately 75–80% of MTCs occur sporadically, while the inherited forms of MTC are responsible for the rest of the cases. The heritable MTC results from a germline mutation in the rearranged during transfection (*RET*) proto-oncogene and is included into the multiple endocrine neoplasia 2 (MEN2), being associated with other endocrine abnormalities and clinical features. MTC is a neuroendocrine tumor that releases a wide range of secretory products that are responsible for a variety of symptoms, making it difficult to be diagnosed. For this reason, the pathological analysis is of vital importance to ensure that the correct diagnosis is made. This review presents the main data from the contemporary literature related to the pathological diagnosis of a patient with MTC and highlights the wide range of tumor cytological features, the many histological variants, as well as the particular tumor immunophenotype. It also reveals the new approach to this type of cancer in the new *World Health Organization* (WHO) *Classification of Thyroid Tumors* (2017) and the reassessment of MTC tumor category in the new *American Joint Committee on Cancer/Tumor, Node, Metastasis* (AJCC/TNM) *Staging* (2017).

Keywords: medullary thyroid carcinoma, sporadic *versus* hereditary, fine-needle aspiration cytology, histological variants, immunohistochemistry.

Introduction

Since the 1990s, the incidence of thyroid cancer has increased faster than any other type of cancer in the United States and other developed countries [1].

A recent study shows that from 1975 to 2009 thyroid cancer presented a three-fold increase in its incidence rates, from 4.9 to 14.3 per 100 000 individuals [2]. This alarming increase in annual incidence may be the effect of some new factors that have appeared in the environment, like radioactivity released by the Chernobyl fallout, in 1986 [3] or an imbalanced selenium intake in people's diet [4, 5]. Some other researchers considered that medullary thyroid carcinoma (MTC) incidence increases due to the development of better diagnostic testing, such as ultra-sonography and fine-needle aspiration biopsy, resulting in an early diagnosis of the disease [6].

Even though thyroid cancer accounts for only 0.5–3% of all malignant diseases, this oncological entity is the most frequent endocrine neoplasia, being responsible for more deaths every year than all the other endocrine malignancies taken together [7].

The major histological types of thyroid cancer are papillary carcinoma (the most frequent type, representing almost 80–85% of all cases), follicular carcinomas (about 10%), medullary carcinoma (5%), and anaplastic carcinoma (less than 5%) [8].

MTC (synonyms: C-cell carcinoma, solid carcinoma with amyloid stroma, parafollicular cell carcinoma) is a malignant epithelial tumor that accounts for only 2–5% of all thyroid malignancies [9], but it is one of the most aggressive types as it is associated with a higher incidence of distant metastasis and poor prognosis [10–12].

Sporadic *versus* hereditary MTC

MTC is a neuroendocrine carcinoma that occurs due to proliferation of calcitonin-producing cells or C-cells, which are also known as parafollicular C-cells.

From an embryological point of view, these cells originate in the neural crest, but they arise in their common location (the upper two-thirds of the thyroid lobes) *via* the ultimobranchial bodies [13].

Approximately 75–80% of MTCs occur sporadically, while the inherited forms of MTC are responsible for the rest of the cases [14–16]. The central roles in the pathogenesis of both hereditary and sporadic forms of MTC are mutations in the rearrangements of rearranged during transfection (*RET*) proto-oncogene, which cause an early oncogenic event that leads to tumorigenesis [17]. The most frequent variants are usually located in exons 10, 11, and 13 through 16 of the *RET* gene [18]. *RET* mutations occur in approximately 50% of patients with sporadic MTC and are associated with poor prognosis compared with the absence of such a mutation [12, 19].

Based on a specific germline mutation in the *RET* proto-oncogene [20], heritable MTC can be a component of three syndromes: multiple endocrine neoplasia 2A (MEN2A) syndrome, multiple endocrine neoplasia 2B (MEN2B) syndrome, and familial medullary thyroid carcinoma (FMTC) syndrome. All three syndromes are autosomal dominant and have variable phenotypic expression and penetrance [21].

The sporadic MTC is usually diagnosed around the age of 60, but the heritable cases are diagnosed at a younger age [22].

The most common subtype of MEN2 is MEN2A, which is responsible for about 95% of all MEN2 cases [16]. Both MEN2A and MEN2B are associated with other endocrine abnormalities and clinical features (Table 1).

Patients with MEN2A syndrome will develop a MTC, often in the second or third decades of life, and will be diagnosed during their life with pheochromocytoma (50% of all MEN2A cases), and with parathyroid hyperplasia (only 25–35% of cases).

Patients with MEN2B will have Marfanoid habitus, and will develop heritable MTC at the youngest age, often under the age of 10. Half of them will be also diagnosed with pheochromocytoma, and eventually will express intestinal ganglioneuromas, mucosal neuromas, ocular abnormalities and musculoskeletal manifestations [16, 23].

Table 1 – ***Clinical manifestations in hereditary MTC syndromes [21, 24]***

Clinical manifestations in MEN syndromes	Prevalence [%]		
	MEN2A	*FMTC*	*MEN2B*
MTC	100	100	100
Pheochromocytoma	10–60	0	50
Parathyroid hyperplasia/ parathyroid adenoma	10–30	0	0
Cutaneous lichen amyloidosis	10	0	0
Marfanoid habitus	0	0	100
Intestinal ganglioneuromatosis	0	0	60–100
Mucosal neuromas (tongue, subconjunctivas)	0	0	70–100

FMTC: Familial medullary thyroid carcinoma; MEN: Multiple endocrine neoplasia; MTC: Medullary thyroid carcinoma; NR: Not reported.

FMTC is characterized by the absence of any extra-thyroidal endocrine tumors, but will develop a MTC during their adult life [12]. Regardless of the type of MTC, sporadic or heritable, female and white persons are affected more frequently [25].

The major prognostic factors of survival in MTC are age and tumoral stage at diagnosis [14, 26]. The 10-year survival rates range from 70% to 90% and 56% to 87% at five years [27]. Patients younger than 40 and those whose tumors are limited to the thyroid gland have a better prognosis than older patients [26, 27]. The 10-year survival rate is 75% for patients with regional spread, decreasing to 40% for those with distant metastases [28].

Clinical characterization of MTC

Usually, upon admission to an Endocrinological Unit, patients present a painless, palpable thyroid nodule during physical examination, often accompanied by cervical adenopathies. The thyroid nodule is associated with clinical symptoms, such as dysphagia, hoarseness, dyspnea and coughing. Moreover, some paraneoplastic symptoms may be present because MTC can release a wide range of ectopic hormones and other substances as well, in various amounts (Table 2).

Table 2 – ***Secretory products of MTC [20, 29, 30]***

Hormones and pro-hormones	Enzymes	Others
Calcitonin	NSE	CEA
ACTH	Histaminase	CgA
β-Endorphin	DOPA-decarboxylase	NGF
β-Melanocyte stimulating hormone	Kinin–kallikrein system	Syn
Somatostatin	–	Neurotensin
Neurotensin	–	Prostaglandin
Cathecolamine	–	Histamine
Substance P	–	Serotonin
Corticotrophin releasing hormone	–	–
Vasoactive intestinal peptide	–	–
Bombesin	–	–
Gastric-releasing peptide	–	–

ACTH: Adrenocorticotropic hormone; CEA: Carcinoembryonic antigen; CgA: Chromogranin A; DOPA: 3,4-Dioxyphenylalanine; MTC: Medullary thyroid carcinoma; NGF: Nerve growth factor; NSE: Neuron-specific enolase; Syn: Synaptophysin.

All the secretory products may cause metabolic disorders and clinical manifestations, such as diarrhea, painful bone metastasis, flushing, or Cushing's syndrome. The biochemical activity of MTC also includes the production of carcinoembryonic antigen (CEA) that, along with calcitonin, are sensitive tumor biomarkers that facilitate the diagnosis of MTC, and their postoperative detection correlates well with tumor relapse or progression being useful in assessing treatment effectiveness [20]. However, calcitonin-doubling times along with large tumor sizes, node metastases, and extrathyroid extension have been identified as prognostic factors for MTC [31].

What can we expect from macroscopic examination in case of a MTC?

First of all, the pathologist has to find the thyroid cancer

and differentiate it from other endocrine pathologies, *i.e.*, C-cell hyperplasia, thyroid adenoma, autoimmune thyroiditis [32–34], as well as from other histological types of thyroid cancer.

MTC is usually located at the junction of the upper and medium thirds of the thyroid lobes, which corresponds to areas where C-cells are usually located [35].

A macroscopic examination of the thyroid gland may point to the type of MTC. Hereditary MTCs are more often multifocal and bilateral, being located in the upper to middle parts of the thyroid lobes [36]. On the other hand, sporadic MTC develops as a unilateral, single, solid, sharply circumscribed, but non-encapsulated tumor, with a dense consistency, and a white-gray to tan color [9, 20]. MTCs can have variable sizes ranging from 0.1 cm in diameter to those that replace the entire thyroid lobe (Figures 1 and 2). The presence of cystic degeneration or area of necrosis is extremely rare.

Pillarisetty *et al.* (2009) suggested that the term medullary thyroid "microcarcinoma" must be applied only for those tumors measuring less than 0.5 mm with a complete absence of metastatic disease or elevated post-operative calcitonin levels [26]. The recent *World Health Organization* (WHO) Classification (2017) defined this pathological entity as a tumor measuring <1 cm in diameter [9].

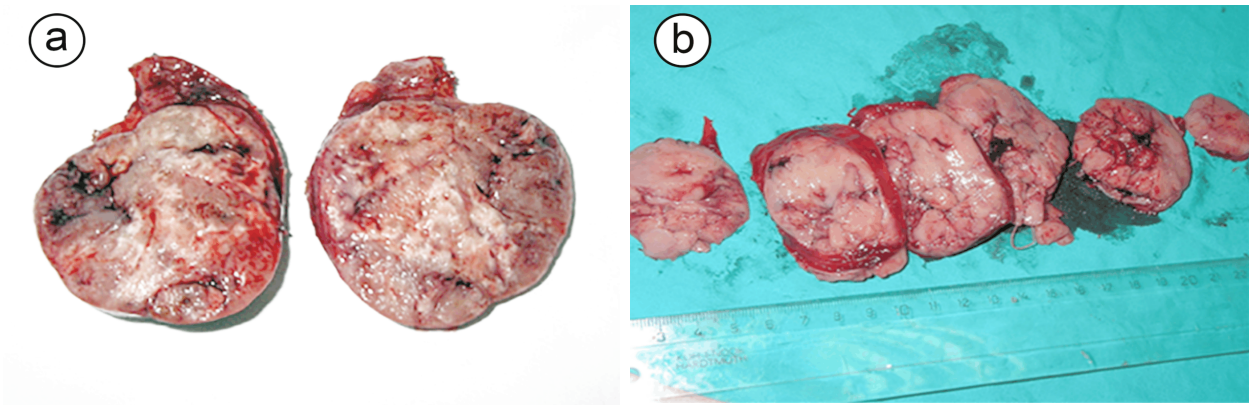

Figure 1 – ***Gross specimens of MTCs: (a) A large, lobulated, solid, and firm grayish mass involving the entire thyroid; (b) A grayish tumor replaced the entire thyroid lobe and measured 3×6 cm. MTC: Medullary thyroid carcinoma.***

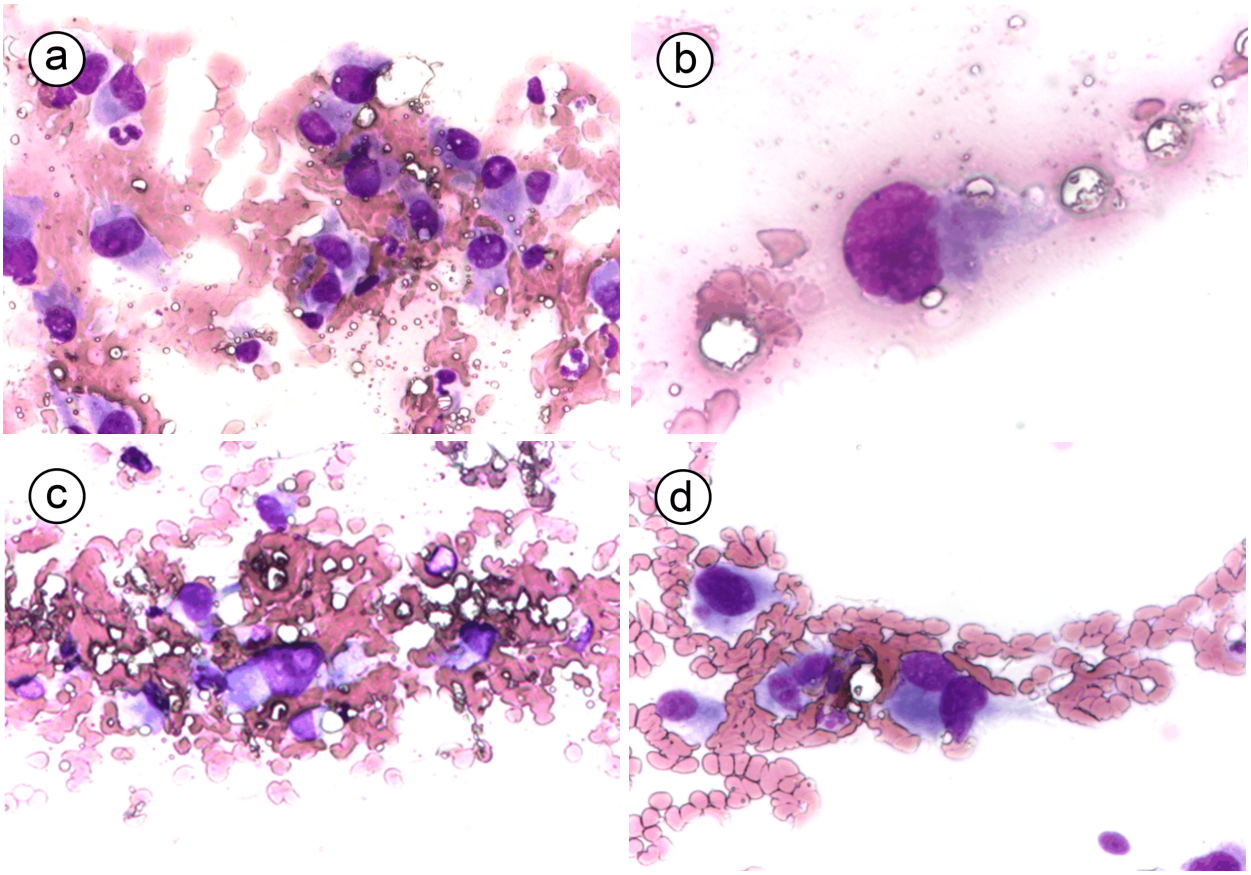

Figure 2 – ***FNAC (MGG staining, ×200): (a) The smear shows a dispersed cellular pattern made up of isolated cells with moderate pleomorphism; (b) Eccentrically placed tumor nucleus presents moderate pleomorphism and "salt and pepper" chromatin – long cytoplasmatic cell processes could be seen; (c) Nuclear pseudoinclusions; (d) The smear exhibits dispersed uni- and bi-nucleated cells with coarsely granular ("salt and pepper") chromatin. FNAC: Fine-needle aspiration cytology; MGG: May-Grünwald–Giemsa.***

Interpretation of the fine-needle aspiration cytology (FNAC) in MTC

FNAC is a useful and safe procedure for the preoperative diagnosis of MTC. It is consider a first line diagnostic test for evaluating of a thyroid lesion, but it is not definitive as the diagnostic accuracy of this method is reduced (76%) probably because this type of cancer is rare and exhibits a wide range of cytological features. Indian researchers obtained a definite cytological diagnosis of medullary carcinoma in 87.1% of cases based on cytomorphology alone and in 12.9% of cases, based on immunocytochemistry for calcitonin [37].

Moreover, in order to establish a definite diagnosis of MTC on FNAC, some Japanese authors proposed calcitonin measurement using needle washout fluid and immunocytochemical staining using anti-calcitonin antibody for diagnosing MTC on FNAC [38].

Cytological smears should be processed and stained by routine Papanicolaou and May-Grünwald–Giemsa (MGG) techniques.

FNAC samples of MTCs represent an adequate cellular aspirate when a dispersed cell pattern could be obtained on the smear. Usually, MTC exhibits a monomorphic cell pattern, or a slight to moderate pleomorphism (Figure 2A). The tumor cells have a polygonal, round, plasmacytoid, and/or spindle-shaped, singly or in clusters. Cytoplasm is basophilic, granular, variable in quantity (moderate to abundant), and, in some cases, long cytoplasmic cell processes can be seen. Tumor nuclei are round, eccentrically placed, with "salt and pepper" chromatin (which represent neuroendocrine nuclear features) (Figure 2B). Occasionally, intranuclear pseudoinclusions (indistinguishable from those seen in papillary carcinoma) can be noticed (Figure 2C). Binucleated and multinucleated cells are usually seen (Figure 2D). Nucleoli are usually discreet. Amyloid deposits may be found in more than half of MTCs. It appears as a dense, amorphous material, in the smear background similar with colloid [39] that stained in blue with Papanicolaou (Pap) staining [40] or variable shades of magenta with MGG. Congo Red staining helps to differentiate amyloid from colloid or hyaline fragments, being diagnostic for MTC [40, 41].

Cytological examination can also highlight the morphological variants of MTCs. Kaushal *et al.* (2011) identified a follicular arrangement in 14.1% of their 78 cases, the melanin production variant, which was a rare event (2/78 cases), the giant cell variant (1/78 cases), with large pleomorphic nuclei and numerous bizarre tumor giant cells, the small cell variant, paraganglioma-like variant and papillary variant (each of them representing only one case from all 78 cases) [37].

Most commonly, MTC is confused on smear with Hürthle cell neoplasm, papillary carcinoma, anaplastic carcinoma, hyalinizing trabecular tumor (HTT), plasmacytoma, and metastatic tumors (particularly melanoma) [42].

New insights into the histopathological examination of MTC

Usually, these tumors are identified on histological sections stained with Hematoxylin–Eosin (HE). At microscopic examination, most sporadic and heritable MTCs appear as well circumscribed but non-encapsulated tumors [43], exhibiting a solid pattern of growth with a wide morphological variety that can mimic any other thyroid malignancy.

The classical MTC shows a lobular, trabecular, insular or sheet-like growth arrangement. Although most of the tumors appear sharply circumscribed at the gross level, microscopic examination often reveals extension of the tumor into the adjacent thyroid tissue.

The prototype cell of MTC is round, polygonal, plasmacytoid, or spindle in shape, with common admixtures of these cell types [27, 44] (Figure 3A). Usually, tumor cells have eosinophilic to amphophilic granular cytoplasm, due to secretory granules [45]. Regarding the nuclei morphology, most of the MTCs present round to oval pattern, with coarsely clumped ("salt and pepper") chromatin, indistinct nucleoli, and occasional nuclear pseudoinclusions. Bi- or multinucleated giant tumoral cells may be identified. In MTCs exhibiting spindle cell morphology, the nuclei are elongated, but the chromatin pattern is still the same [43]. Commonly, these tumors present only mild nuclear pleomorphism, and mitotic activity is low (Figure 3B). In small MTCs foci of necrosis, hemorrhage, and mitotic activity are uncommon, while in larger tumors they are frequent [44, 46, 47].

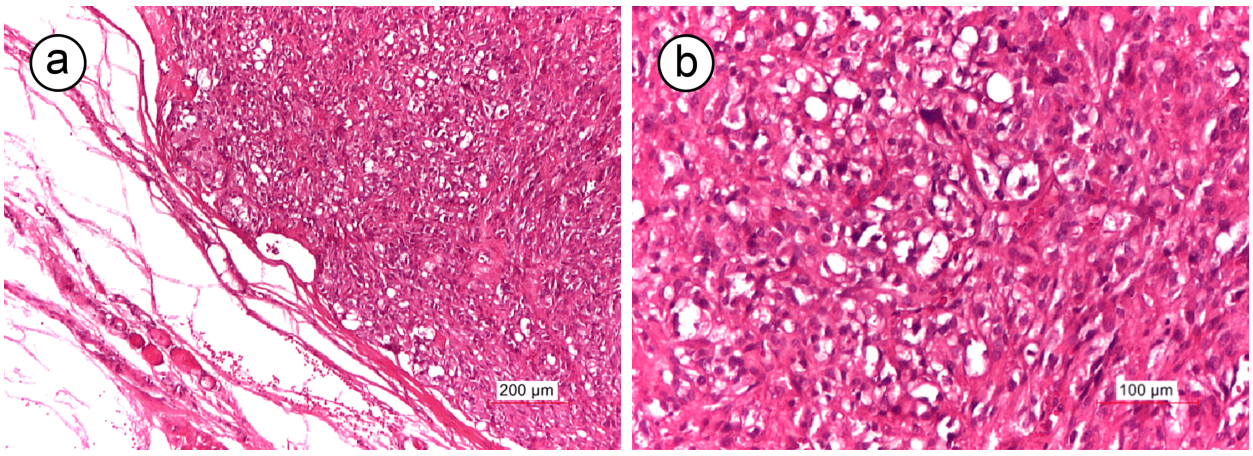

Figure 3 – ***Histological sections of MTC: (a) Tumor is made up of epithelioid and fusiform cells; (b) The tumor nuclei exhibit a moderate degree of variation in size, and low mitotic activity. HE staining: (a) ×40; (b) ×100. HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

In up to 90% of MTC cases, stroma contains amyloid deposits (Figure 4A), having procalcitonin and calcitonin as major constituents. Being unevenly distributed throughout the tumor, amyloid appears as a Congo Red-positive material (Figure 4B), with typical "apple-green" birefringence (Figure 4C) when examined in polarized light [48]. In histological sections stained with Crystal Violet, the amyloid deposits are typically metachromatic. Massive coarse deposition of amyloid form interlacing trabeculae between the tumor cells, separating them into irregular sheets. Thin amyloid deposits can lead to pseudopapillary pattern as tumor cells arrange around these fiber-like structures of eosinophilic material.

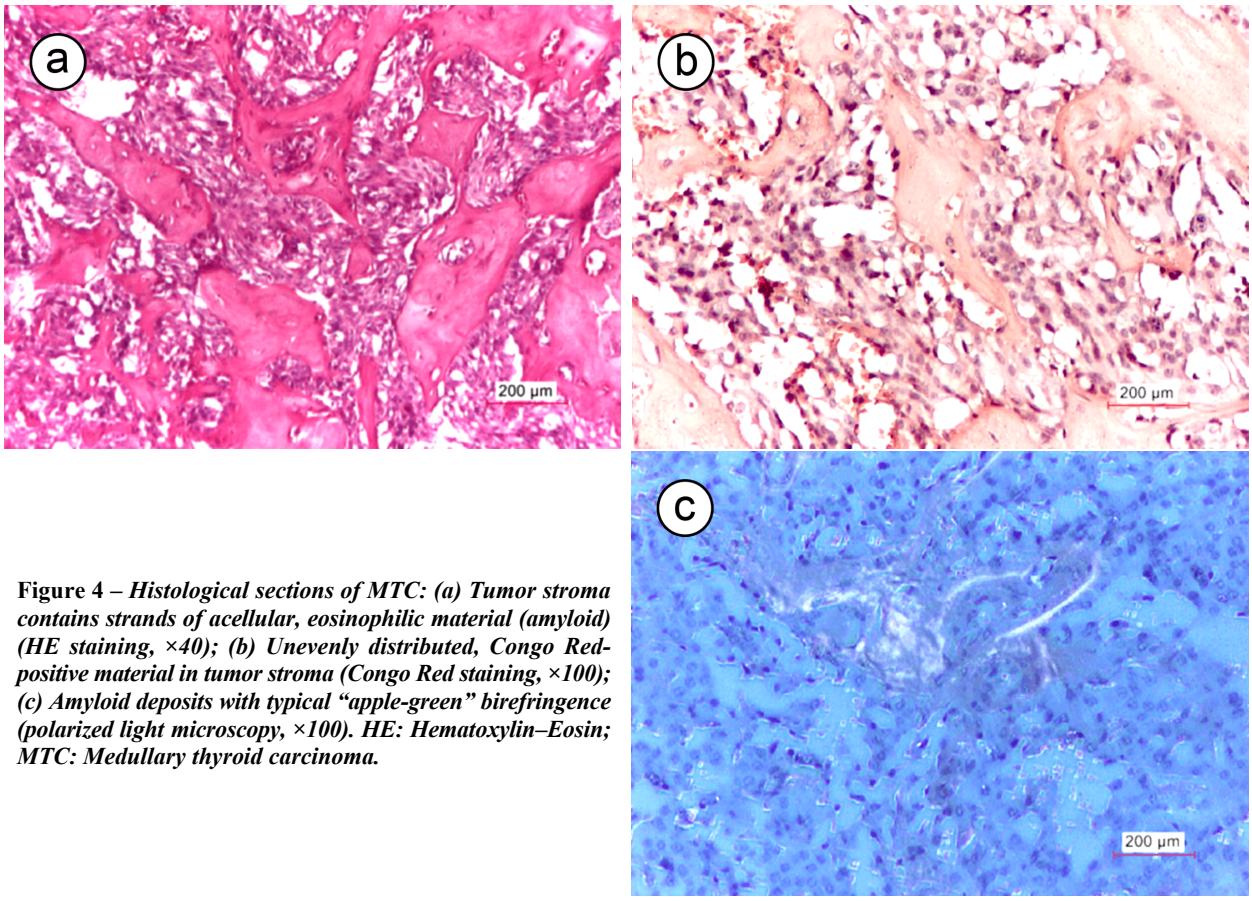

Figure 4 – ***Histological sections of MTC: (a) Tumor stroma contains strands of acellular, eosinophilic material (amyloid) (HE staining, ×40); (b) Unevenly distributed, Congo Red-positive material in tumor stroma (Congo Red staining, ×100); (c) Amyloid deposits with typical "apple-green" birefringence (polarized light microscopy, ×100). HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

In addition to amyloid, stroma can also contain variable amounts of collagen and a prominent vascularity with glomeruloid configuration or long cords of vessels, coarse calcifications (Figure 5), and psammoma-like bodies [49].

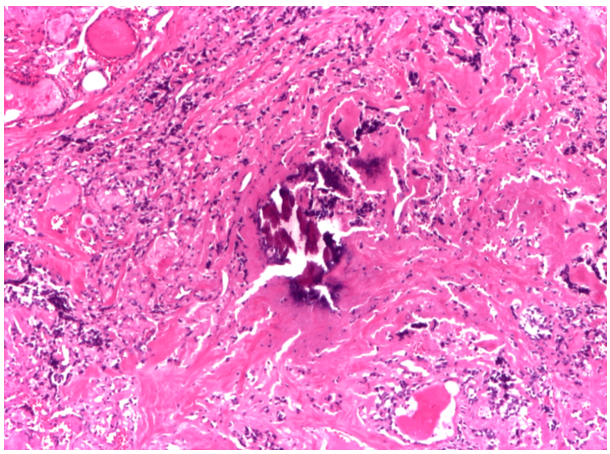

Figure 5 – ***Histological section of MTC: stroma with variable amounts of collagen and calcifications (HE staining, ×40). HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

From a histological point of view, heritable MTCs are virtually indistinguishable from those exhibited by sporadic tumors, but there are researchers found some differences. Kaserer *et al.* (2001) reported that hereditary MTCs present desmoplastic stroma and are prone to metastasizing into the regional lymph nodes [22].

Diaz-Cano *et al.* (2001) highlighted the fact that heritable MTCs are accompanied by C-cell hyperplasia [50]. However, this histological feature is not an absolute diagnostic criterion for MTC. Taking all MTCs together, irrespective of the fact that the tumor is sporadic or heritable, Desai *et al.* (2005) reported that the thyroid gland adjacent to a MTC can exhibit a normal appearance in more than half of the cases, but in the rest of the cases a chronic autoimmune thyroiditis, or even a second tumor, *i.e.*, a papillary thyroid carcinoma, could be found [46].

The many "histological faces" of MTC

In the last three decades, the spectrum of morphological features observed in MTC enlarged with many new entities. Numerous variants of microscopic patterns were noted in MTCs. The pathologists should be aware of all these variants in order to establish the correct

diagnosis. Facing a rare histological variant of MTC, immunohistochemical (IHC) stainings (especially positivity for calcitonin and CEA to confirm a MTC), but also the presence of amyloids are essential.

MTCs include many histological variants under the same "umbrella", such as: encapsulated, follicular, pseudopapillary, oncocytic, squamous, with rosette formation, with small cells, with clear cells, with melanin pigmentation, with giant cells, amphicrine and paraganglioma-like type [9, 34, 51–61].

In what follows, we present the histological subtypes of MTC that were diagnosed and published so far in the literature:

(*a*) The encapsulated subtype defined a thyroid tumor that surrounded by a complete fibrous capsule [9].

(*b*) The follicular/glandular subtype is made up of tumor cells that form follicular or glandular structures containing eosinophilic secretion in the lumens (Figure 6). The endoluminal cytoplasm of tumor cells is often more deeply eosinophilic and granular because of the accumulation of neurosecretion granules at this site, especially chromogranin A (CgA) [53].

(*c*) The oncocytic/oxyphilic subtype exhibits tumor cells with abundant eosinophilic granular cytoplasm that are arranged in nests or in a trabecular fashion, in a focal or a diffuse pattern (Figure 7). Neoplastic oncocytic foci are immunoreactive for calcitonin, galectin-3 and thyroid transcription factor 1 (TTF1). This subtype develops at an older age (almost 64 years) and has a stronger predominance in women [9, 53, 54].

(*d*) The pseudopapillary subtype defines a tumor made up of pseudopapillae formed by tissue fragmentation, but rarely even true papillae can occur [9, 53] (Figure 8, A and B).

(*e*) Small cells subtype of MTC is a rare variant and an aggressive form, made up of diffusely infiltrating small blue round cells with scanty cytoplasm and inconspicuous nucleoli. The diagnosis is based on the identification of the amyloid deposits, using van Gieson and Congo Red stainings (Figure 9, A–D). The tumor cells are strongly positive for cytokeratin (CK) AE1/AE3 and CEA, focally for CK7, synaptophysin (Syn), cluster of differentiation (CD) 56, and CD99 (Ewing's sarcoma marker – MIC2), while negative for calcitonin, p63, p40, and CgA [34].

(*f*) Squamous variant of MTC presents focal squamous differentiation, but this is an exceptional finding [9, 55].

(*g*) Clear cell variant of MTC reveals cells with optically clear cytoplasm, which dominate the picture of the tumor or can appear only focally [9, 53].

(*h*) Melanin-producing/pigmented/melanotic variant can be found only in rare cases of MTC, exhibiting cells containing variable amounts of melanin pigment in their cytoplasm, but tumor cells also express strong positive reaction to calcitonin by IHC staining. Melanin pigment could be also found in the extracellular matrix of the tumor [53, 56, 57].

(*i*) MTC variant with giant cells shows intermingled pattern of typical small cells with giant, large cells, with bizarre and pleomorphic nuclei, with nuclear pseudoinclusions, sometimes even multinucleated cells (Figure 10). Mitoses can be absent or very few, but stromal amyloid is identified. IHC stainings reveal positive reactivities for calcitonin and CEA, but negativity for thyroglobulin. Ki67 labeling index is very low (less than 1%) and as such, this subtype has a good prognosis [9, 53, 58].

(*j*) Amphicrine/mucin-producing/mucinous variant is identified only in rare cases. It is characterized by tumor cells that contain mucin and express calcitonin. Tumoral stroma contains extensive mucin secretion and focal areas of Congo Red-positive amyloid [9, 59].

(*k*) Paraganglioma-like type shows nested architecture delineated by delicate vasculature, mimicking paraganglioma. S100 protein-positive sustentacular-like cells are interspersed [9, 53].

(*l*) Angiosarcoma-like variant of MTC is a tumor with pseudosarcomatous features resembling those of angiosarcomas [9].

(*m*) Spindle cell variant of MTC is entirely made up of plump spindle cells arranged in intersecting fascicles, whorls, and packets, mimicking mesenchymal neoplasms, but amyloid-like material is abundant in the background (Figure 11). Nuclear pleomorphism is mild and mitotic count is very low [53, 60].

(*n*) Carcinoid-like variant of MTC shows histological features resembling intestinal carcinoid, with tumor islands, trabeculae, or glands separated by fibrohyaline stroma [53].

(*o*) Neuroblastoma-like/with rosette formation variant is also a rare subtype of MTC and exhibits a fibrillary matrix and rosettes, resembling neuroblastoma [53, 61].

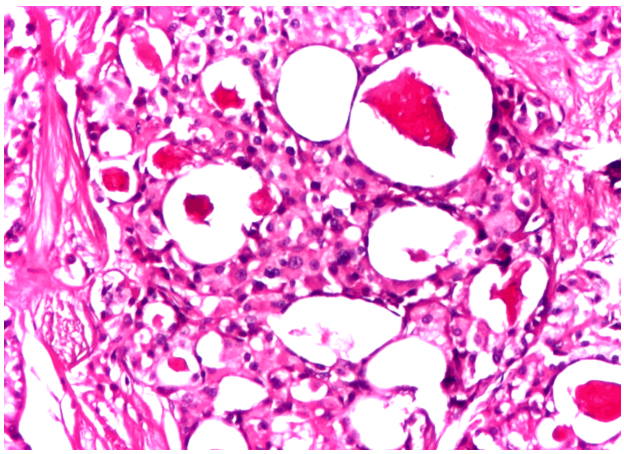

Figure 6 – ***Histological sections of MTC: follicular subtype (HE staining, ×100). HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

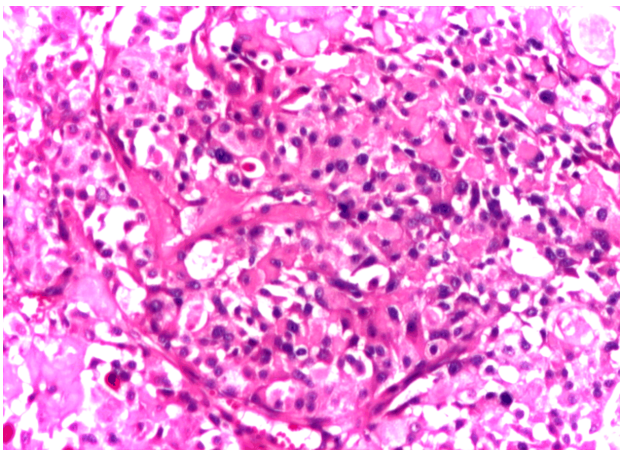

Figure 7 – ***Histological sections of MTC: oncocytic subtype (HE staining, ×100). HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

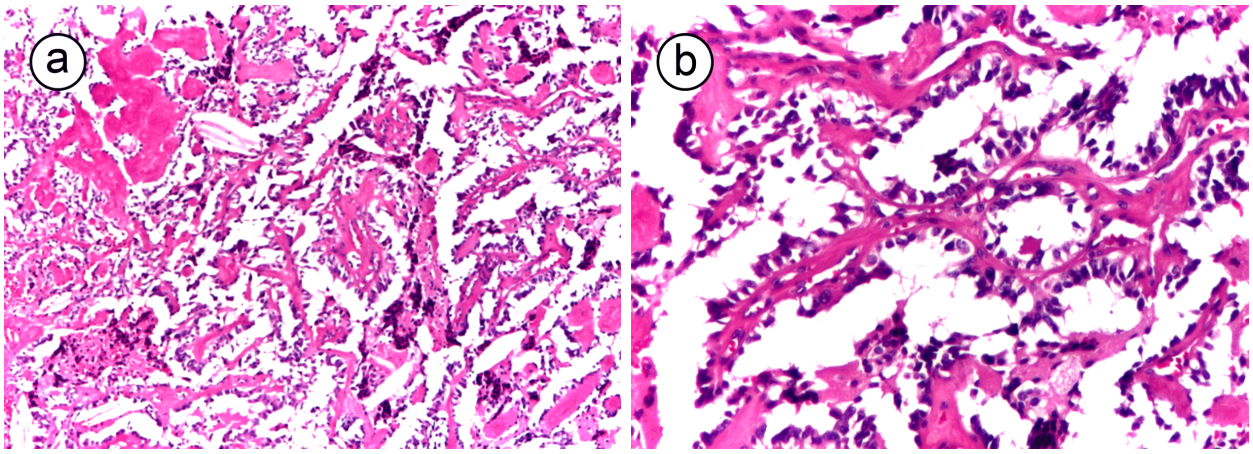

Figure 8 – ***Histological sections of MTC – pseudopapillary subtype: (a) Tumor cells are arranged around thin amyloid deposits in a pseudopapillary pattern; (b) Higher magnification exhibits the area where shrinkage has led to pseudopapillary arrangement of the tumor cells around the amyloidotic "core". HE staining: (a) ×40; (b) ×100. HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

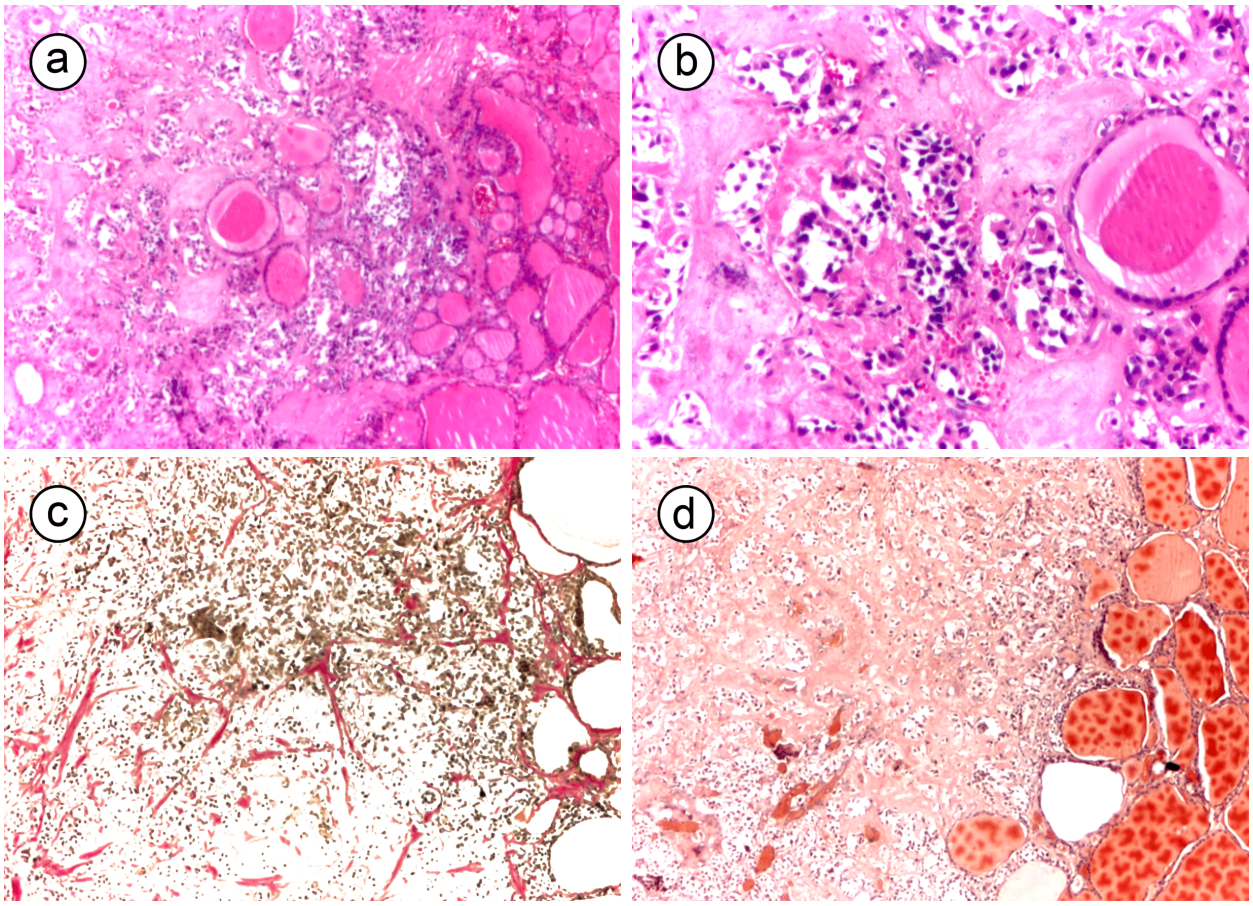

Figure 9 – ***Histological sections of MTC – small cells subtype: (a) Small tumor cells infiltrate between the thyroid follicles from the vicinity; (b) Higher magnification reveals small tumor cells and entrapped follicles; (c) Small tumor cells are embedded into amyloid deposits (Van Gieson staining, ×40); (d) Congo Red positive amorphous extracellular substance (amyloid deposits) (Congo Red staining, ×40). HE staining: (a) ×40; (b) ×100. HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

All the histological variants listed above seem to have no clinical or prognostic significance, except the small cell type, which is more aggressive. The prognostic implication of the giant cell variant is still unsettled [61]. In all cases exhibiting one of these histological subtypes of MTC, the correct diagnosis should take into consideration other thyroid pathological entities with which they are alike (Table 3).

How important are histochemical stainings in MTC?

Regarding the histochemical stainings that could be applied in case of MTCs, some studies reported that 90% of these cancers show positivity to Grimelius' argyrophil silver staining. Tumor cells exhibit a weak to moderate argyrophilia, but a strong positivity could be occasionally

obtained in dispersed cells [62]. Moreover, the tumor cells may also show focal PAS and Alcian Blue reactivity. The PAS positivity does not appear to be related to glycogen even in those tumors with a clear cytoplasm [46].

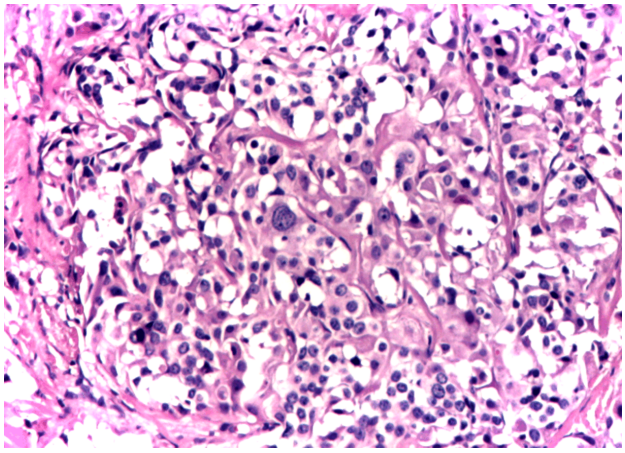

Figure 10 – ***Histological sections of MTC – giant cells subtype: the tumor is made up of giant large cells, with bizarre and pleomorphic nuclei intermingled with typical small cells (HE staining, ×100). HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

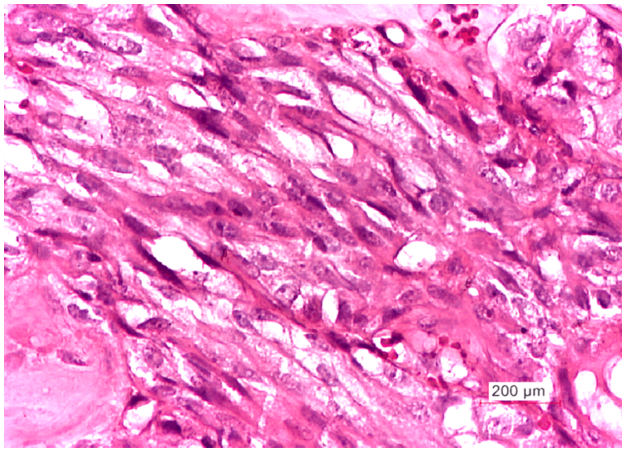

Figure 11 – ***Histological sections of MTC – spindle cell subtype: the tumor exhibits plump spindle cells with prominent nucleoli that are arranged in intersecting fascicles, mimicking mesenchymal neoplasms; amyloid-like material can be identified in the background (HE staining, ×200). HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

Table 3 – ***Histological subtypes/variants of MTC and their DDx [9, 53–61]***

Histological variant of MTC	Thyroid pathology that should be taken into consideration in DDx
Encapsulated	–
Follicular/glandular	Follicular adenoma or carcinoma; poorly differentiated thyroid carcinoma
Pseudopapillary	Papillary thyroid carcinoma
Oncocytic/oxyphilic	Hürthle cell adenoma and carcinoma
Squamous variant	Primary or metastatic squamous cell carcinoma of thyroid gland
Small cells variant	Malignant lymphoma of the thyroid; thyroid metastasis of a small cell lung carcinoma
Clear cell variant	Other clear cell tumors
Melanin-producing/ pigmented/melanotic variant	–
With giant cells	Undifferentiated carcinoma
Amphicrine variant/ mucin-producing/mucinous	–
Paraganglioma-like type	Paraganglioma
Angiosarcoma-like variant	–
Spindle cell	Mesenchymal tumors
Carcinoid-like	Metastatic carcinoid; paraganglioma; follicular neoplasm
Neuroblastoma-like/ with rosette formation	Malignant lymphoma; metastatic neuroblastoma; peripheral primitive neuroectodermal tumor

DDx: Differential diagnosis; MTC: Medullary thyroid carcinoma.

The value of immunohistochemistry in MTC

By having so many histological variants, MTC represents the great mimicker of other thyroid cancers. In order to establish a correct diagnosis, immunohistochemistry should be performed in all difficult cases.

A wide variety of substances can be demonstrated immunohistochemically in MTC (Table 4), but a relatively small number have practical diagnostic or prognostic value. The most useful diagnostic stainings include calcitonin, CEA, CgA, and Syn [63].

Table 4 – ***The "typical" immunophenotypic profile of MTC [53, 63, 64]***

Usually positive immunostaining	Variable immunopositivity	Usually negative immunostaining
CgA	S100 protein	Thyroglobulin
NSE	Vimentin	CK20
Syn	–	–
CD56	–	–
CK AE1/AE3	–	–
CK7	–	–
TTF1	–	–
Calcitonin	–	–
CEA	–	–

CD: Cluster of differentiation; CEA: Carcinoembryonic antigen; CgA: Chromogranin A; CK: Cytokeratin; MTC: Medullary thyroid carcinoma; NSE: Neuron-specific enolase; Syn: Synaptophysin; TTF1: Thyroid transcription factor 1.

Duan & Mete (2016) proposed an IHC algorithm that should be used in the diagnosis of a MTC, when the pathologists suspected a neuroendocrine tumor [65]. According to them, the following antibodies must be used, in a well-established order: (*i*) markers for neuroendocrine differentiation [CgA, Syn, CD56, CD57, protein gene product 9.5 (PGP9.5), and neuron-specific enolase (NSE)]; (*ii*) markers for epithelial differentiation: CKs AE1/AE3 and CAM5.2; (*iii*) marker for tumor proliferation: Ki-67/MIB-1; (*iv*) markers for site of origin: TTF1, monoclonal CEA, and calcitonin.

MTC cells show positivity for low-molecular-weight CK proteins; high-molecular-weight CK is rarely expressed in this cancer. MTCs also exhibit positive staining with antibodies directed to neuroendocrine products, but also

to CEA, all of them being secreted by tumor cells. As such, positive results are obtained with anti-NSE, anti-CgA (Figure 12A), and anti-Syn (Figure 12B) antibodies. More specific is the positive IHC staining with anti-calcitonin antibody (Figure 12C), which can be identify in approximately 80% of cases [27, 66], thus confirming the involvement of parafollicular calcitonin-producing C-cells. Moreover, immunostaining of tumor C-cells for calcitonin further helps to identify tumor angioinvasion and extracapsular spreading of tumor cells.

Tumor cells are also characteristically immunopositive for CEA, an antigen that is not usually expressed by thyroid neoplasms derived from follicular cells, and for TTF1 (Figure 12D), but not for thyroglobulin (Figure 12E). The presence of thyroglobulin positivity reflects the presence of entrapped non-neoplastic follicles or a neoplastic follicular component in a mixed medullary and follicular cell carcinoma.

MTCs express immunopositivity for a number of other neuropeptides, such as somatostatin, gastrin-releasing peptide, adrenocorticotropic hormone (ACTH), substance P, vasoactive intestinal peptide, and cathecolamine [64].

It is worth mentioning that medullary thyroid microcarcinomas could be observed as an incidental finding of the pathologist in searching for another histopathological entity in an excised thyroid gland (Figure 13, A and B).

Furthermore, the diagnosis of a MTC could be very difficult to establish only on HE-stained sections of patients with undetectable levels of serum calcitonin, but in almost half of these cases immunohistochemistry can detect diffuse or focal positivity for calcitonin, CgA and CEA [35]. However, Gambardella *et al.* (2019) and Zhou *et al.* (2017) found out that this type of MTC has a better oncological outcome than a calcitonin-rich MTC [35, 67].

TTF1 is positive in at least 80% of medullary carcinomas, and thyroglobulin is negative in MTC cells, but is positive in residual entrapped normal follicles [68].

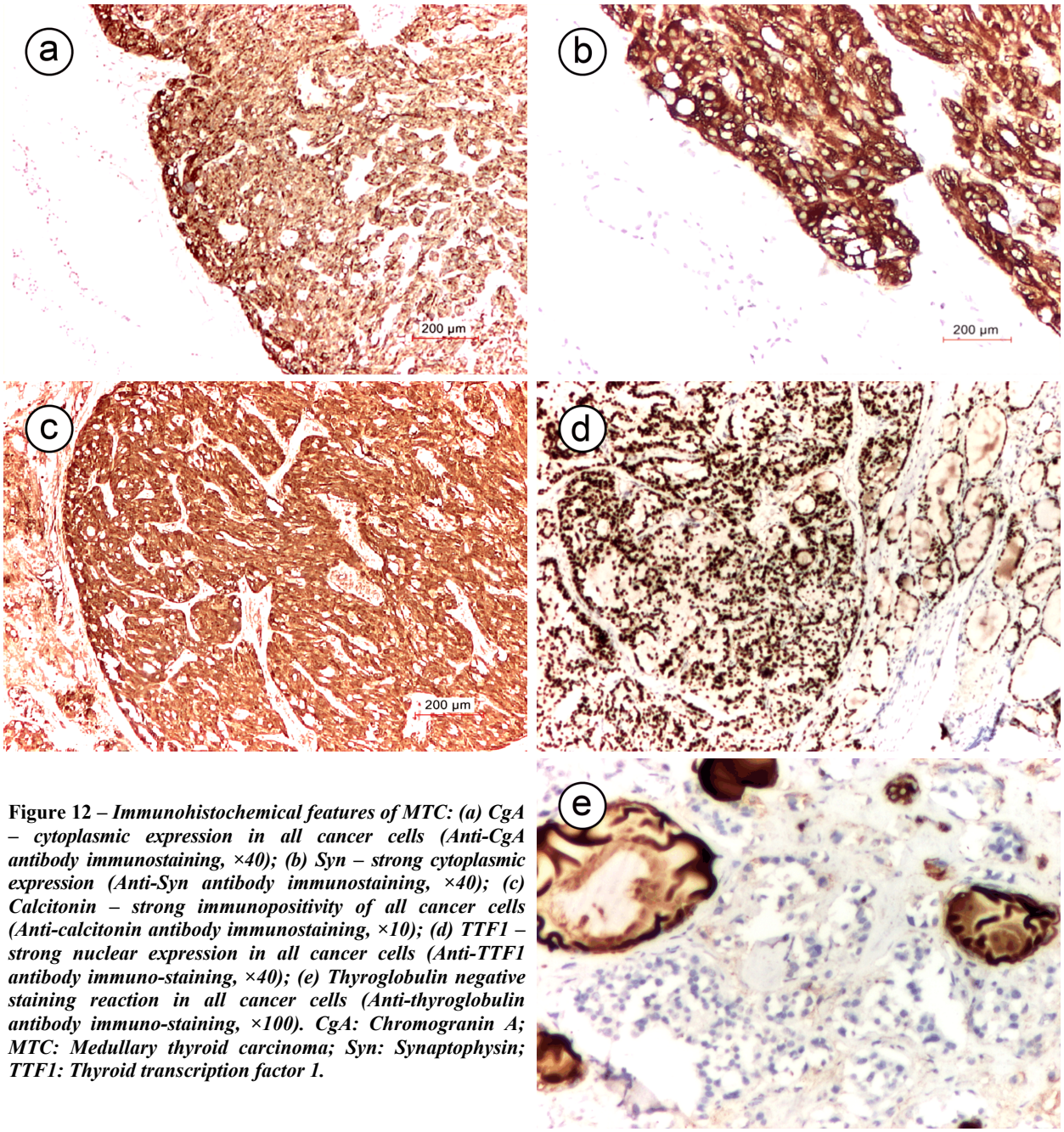

Figure 12 – ***Immunohistochemical features of MTC: (a) CgA – cytoplasmic expression in all cancer cells (Anti-CgA antibody immunostaining, ×40); (b) Syn – strong cytoplasmic expression (Anti-Syn antibody immunostaining, ×40); (c) Calcitonin – strong immunopositivity of all cancer cells (Anti-calcitonin antibody immunostaining, ×10); (d) TTF1 – strong nuclear expression in all cancer cells (Anti-TTF1 antibody immuno-staining, ×40); (e) Thyroglobulin negative staining reaction in all cancer cells (Anti-thyroglobulin antibody immuno-staining, ×100). CgA: Chromogranin A; MTC: Medullary thyroid carcinoma; Syn: Synaptophysin; TTF1: Thyroid transcription factor 1.***

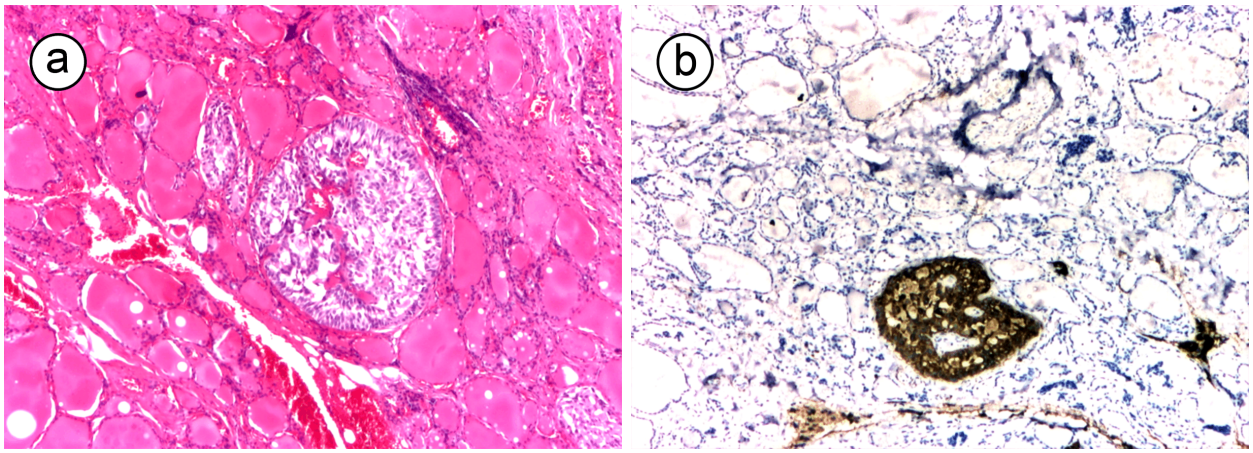

Figure 13 – ***Medullary thyroid microcarcinoma, as an incidental finding: (a) The tumor has very small dimensions, is surrounded by thyroid hyperplasia and its amyloid deposits are very scant (HE staining, ×40); (b) Tumor cells express strong immunopositivity for anti-Syn antibody (immunostaining, ×40). HE: Hematoxylin–Eosin; Syn: Synaptophysin.***

Serum calcitonin and CEA levels are usually increased in patients with MTC, compared to patients with papillary, follicular, or anaplastic carcinomas, and correlate with calcitonin and CEA positivity immunostaining (100%) of tumor cells. Aggressive cases of MTCs show persistent elevated CEA levels, but decreased calcitonin serum levels.

Usually, MTC expresses low values of Ki67 labeling indexes (Figure 14). Higher Ki67 labeling indexes are associated with extra-thyroid spread, with lymph nodes and distant metastases, advanced stage, and low overall survival, the cut-off expression being over 50 cells/mm^2 [47, 69].

Current updates on metastases of MTC

MTC frequently metastasizes and the tumor spreads occur both by hematogenous and lymphatic way. As such, lymphatic and vascular invasion may be seen at the advancing front of the tumor (Figure 15).

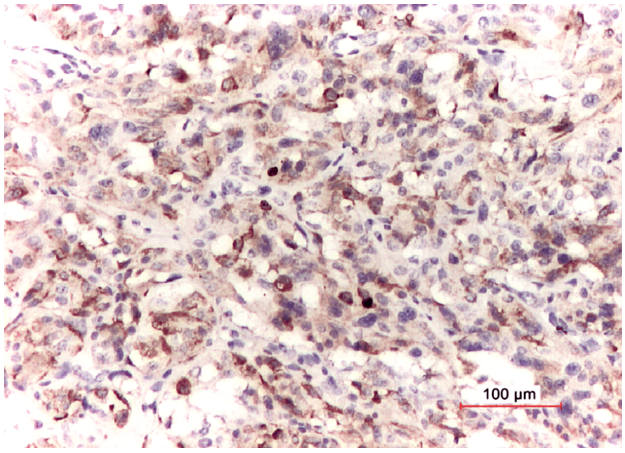

Figure 14 – ***Proliferative activity in MTC: strong nuclear immunopositivity for Ki67, but only in very few scattered tumor cells (Anti-Ki67 antibody immunostaining, ×100). MTC: Medullary thyroid carcinoma.***

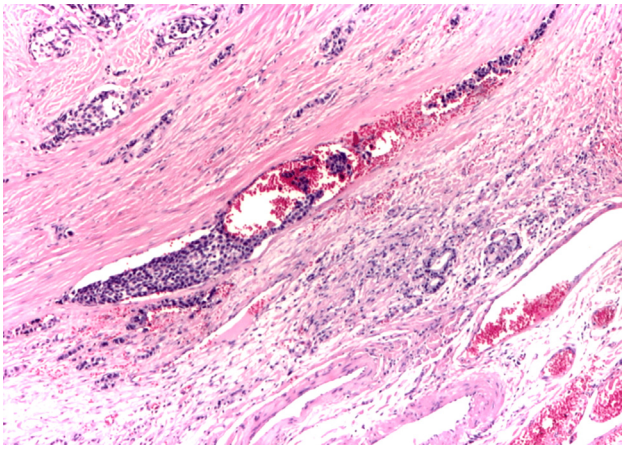

Figure 15 – ***Hematogenous dissemination of MTC: clusters of tumor cells in endothelium-lined spaces also containing red blood cells (HE staining, ×40). HE: Hematoxylin–Eosin; MTC: Medullary thyroid carcinoma.***

Regional lymph nodal involvement occurs early in the evolution of MTCs and affects almost 60% of patients in the moment of their diagnosis [70]. Especially the neck nodes are affected, *i.e.*, the nodes of the central compartment, tracheal and paratracheal nodes, but also the mediastinal nodes [46].

In advanced cases, foci of lymphatic invasion may be seen in the contralateral lobe [27].

During the evolution of MTC, distant metastases can be found in almost 40% of patients [70].

Zhou *et al.* (2017) found out that larger tumoral masses were associated with higher rate of lymph nodes metastasis, and considered that tumor size represents an independent survival indicator [67].

Metastases to the cervical lymph nodes are detected in 70% of cases of MTC. Usually, distant tumor metastases could be detected into the lung, liver, bones and mediastinum [23, 46, 71].

Probably, due to longer survival of the patients with MTCs, some unusual metastasizing sites were recently published. Recently, Sastry *et al.* (2018) reported a rare case of a MTC metastasizing to the brain 21 years after the thyroidectomy [72]. Tanwar *et al.* (2018) also published an article regarding an even more unusual metastasis of MTC. They reported breast metastases from MTC and the case represented a real challenge for the clinician, radiologist, and pathologist as MTCs can mimic primary invasive lobular carcinoma of the breast, both histopathologically and radiologically [70].

Gajdzis *et al.* (2018) reported a patient who had

undergone thyroidectomy for MTC, but 33 years later, he was diagnosed with parotid and intraocular choroidal metastases of his earlier thyroid cancer [73].

New *WHO* Classification (2017) and *AJCC/TNM* Staging (2017) of thyroid tumors brought new reassessments in MTC

The new *WHO Classification* of thyroid tumors released in 2017 is the result of a realistic review of thyroid neoplasms in concordance with the genetic–molecular characterization of these neoplasias. The new edition has significantly expanded upon the information, compared to the previous edition, referring to familial medullary and non-medullary thyroid cancer, including both the syndromic tumor types and those unassociated with tumors in other locations [9, 32, 74].

In the new edition of *American Joint Committee on Cancer/Tumor, Node, Metastasis* (AJCC/TNM) *Staging*, also released in 2017, there is a reassessment of the tumor category in correspondence with the interest of the perithyroid adipose tissue. Thus, the T3 category in the old classification, corresponding to the tumors with any dimensions with minimum perithyroid extension in the adipose tissue, is established in the new edition in a new category. The T3 tumor group addressed only tumors that interest the muscular structures (such as sternohyoid, sternothyroid or omohyoid muscles), considering today that nowadays, adipose tissue can be found discontinuously in the capsule of the organ and did not necessarily represent an external capsule for tumor extension.

Both micro- and macrometastasis in lymph nodes define the pathological N1 group. Although there is no specific number of lymph nodes to be sampled for the definition of the metastatic disease, it is suggested to record prognostic factors as classified under the number of lymph nodes sampled and involved, extranodal involvement, size of the lymph nodes containing metastatic disease and also the size of the metastatic focus [75].

Because the assessment of N/M status may be impossible until the surgical resection is performed, refinement of the N/M status should be done using the identification of the metastatic disease in a period of 12 to 16 weeks after thyroid surgery. Even if the cancer progresses or recurs, the formal stage established during the first four months of follow-up does not change over time, a fact which is also consistent with the *AJCC* staging rules. The stage of the cancer may be reconsidered during follow-ups as new data becomes available by using the same approach and definitions as in the initial staging. This kind of restaging is marked with the lowercase letter "r".

Few of the most important pathological factors in the prognosis include tumor pattern, necrosis, amyloid content and mitotic activity. There is usually a better prognosis in cases presenting encapsulated tumors, with abundant amyloid deposit and uniform cytology, but the significance of any of these variables is uncertain (in multivariate analysis), and as such, these elements were not incorporated into the primary staging scheme [75]. The staging of MTC does not use the histological grade (G) [75].

Conclusions

Prognosis of MTC has greatly improved due to the use of a wide variety of diagnosis methods. Immunohistochemistry is a highly relevant evaluation method, because it can provide crucial information for clinical decisions that influence the outcome. The prognostic significance of the histological features and of immunophenotype in MTC and their impact on therapy are still subject of debate.

Conflict of interests

The authors declare that they have no conflict of interests.

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