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Papel da calcitonina pós-operatória no manejo do carcinoma medular de tireoide.

Porto Alegre
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- Artigo de revisão: Medullary thyroid carcinoma beyond surgery: advances, challenges, and perspectives.

  Lucieli Ceolin; Marta Amaro da Silveira Duval; Antônio Felippe Benini; Carla Vaz Ferreira and Ana Luiza Maia.

  Endocrine-Related Cancer. v.26, p.499 - 518, 2019.Impact factor: 4.8.

- Artigo original: Undetectable postoperative calcitonin is a strong predictor of long-term disease-free survival in medullary thyroid carcinoma.
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Undetectable postoperative calcitonin as a prognostic marker for long term disease-free survival im MTC patients: A cohort analysis.

Trabalho vencedor do Prêmio Jovem Cientista (Apresentação oral)

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Avaliação da calcitonina pós-operatória indetectável com preditor de cura em pacientes com carcinoma medular de tireoide.

- XVIII Encontro Brasileiro de Tireoide, 2018, Campos do Jordão/SP

Calcitonina pós-operatória como fator prognóstico em pacientes com carcinoma medular de tireoide.

- ENDO 2018, Chicago/EUA.

Postoperative Calcitonin As Prognostic Factor In Medullary Thyroid Cancer

Além dos artigos já citados, ao longo do período do doutorado foram desenvolvidos os seguintes trabalhos:

- Tratamento do Carcinoma Medular de Tireoide. Marta Amaro da Silveira Duval e Ana Luiza Maia. Endocrinologia Clínica, sétima edição, Lucio Vilar, 2021.

- Laura Marmitt, Marta A. D. S. Duval, André B. Zanella, Carla V. Ferreira, Antônio F. Benini, Marli V. Camelier, Lucieli Ceolin, Ana Luiza S. Maia. Impacto do rastreamento genético em crianças e adolescentes com neoplasia endócrina múltipla tipo 2: vinte anos de experiência de um centro de referência. 13 Congresso de Endocrinologia e Metabologia da Região Sul, 2019, Gramado/RS.

- Duval MADS, Zanella AB, Cristo AP, Faccin CS, Graudenz MS, Maia AL. Impact of Serum TSH and Anti-Thyroglobulin Antibody Levels on Lymph Node Fine-Needle Aspiration Thyroglobulin Measuremnts in Differentiated Thyroid Carcinoma Patients. European Thyroid Journal, v. 6, p. 292-297, 2017.

LISTA DE ABREVIATURAS E SIGLAS

ARTN Artemin

CA 19.9 Carbohydrate Antigen

CEA Carcinoembrionic Antigen

CLA Cutaneous Lichen Amyloidosis

C-MET Hepatocyte Growth Factor

DRS Dynamic Risk Stratification

DT Doubling Time

EBRT External Beam Radiation Therapy

FMTC Familial Medullary Thyroid Carcinoma

FNA Fine Needle Aspiration

GDNF Glial Cell Line Derived Neurotrophic Factor

HCPA Hospital de Clínicas de Porto Alegre

HD Hirschsprung´s Disease

HGF Hepatocyte Growth Factor

HPT Hyperparathyroidism

INCA Brazilian National Cancer Institute

LD Linkage Disequilibrium

LN Lymph Node

MEN2 Multiple Endocrine Neoplasia Type 2

MKI Multikinase Inhibitors

MTC Medullary Thyroid Cancer

NRTN Neurturin

OS Overall Survival

ORR Objective Response Rate

PDK1 Pyruvate Dehydrogenase Kinase Isozyme 1

PDL1 Programmed Death Ligand 1

PFS Progression-free Survival

PHEO Pheochromocytoma

POCal Postoperative Calcitonin

PSPN Persephin

RET REarranged during Transfection

ROC Receiver Operating Characteristic

TKI Tyrosine Kinase Inhibitors

TSH Thyrotropin

US Ultrasound

VEGF Vascular Endothelial Growth Factor

SUMÁRIO

Parte I

MEDULLARY THYROID CARCINOMA BEYOND SURGERY: ADVANCES, CHALLENGES, AND PERSPECTIVES

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Medullary Thyroid Carcinoma beyond Surgery: Advances, Challenges, and Perspectives.

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ABSTRACT

Medullary thyroid carcinoma (MTC) is a rare type of tumor that originates from thyroid C-cells and accounts for 2-4% of all malignant thyroid neoplasms. MTC may occur sporadically or be inherited, as part of the MEN 2 syndrome. Germ-line mutations of the *RET* (REarranged during Transfection) proto-oncogene cause hereditary cancer, whereas somatic *RET* mutations and, less frequently, *RAS* mutations have been described in sporadic MTC samples. Since early surgery with complete resection of tumor mostly determines the likelihood of attaining cure for MTC, the broader use of *RET* genetic screening has dramatically changed the prognostic of gene carriers in hereditary MTC. Nevertheless, despite recent advances, the management of advanced, progressive MTC remains challenging. The multikinase inhibitors (MKI), vandetanib and cabozantinib, were approved for the treatment of progressive or symptomatic MTC, and several other compounds have exhibited variable efficacy. Although these drugs have been shown to improve progression-free survival, no MKI has been shown to increase overall survival. As these drugs are nonselective, significant off-target toxicities may occur, limiting achievement of the required TK-specific inhibition. Recently, next-generation small-molecule TKI has been developed. These TKI are specifically designed for highly potent and selective targeting of oncogenic *RET* alterations, making them promising drugs for the treatment of advanced MTC. Here, we summarize the current understanding of the intracellular signaling pathways involved in MTC pathogenesis as well as the therapeutic approaches and challenges for the management of advanced MTC, focusing on targeted molecular therapies.

INTRODUCTION

Medullary thyroid carcinoma (MTC) is a malignant tumor originating in parafollicular or C-cells of the thyroid. The main secretory product of MTC is calcitonin, a specific and highly sensitive biomarker that is produced by normal and neoplastic C-cells. Neoplastic C-cells also produce the carcinoembryonic antigen (CEA). These molecules are widely used markers for the diagnosis, prognosis, and follow-up of MTC patients.

The overall frequency of MTC is not well established, but it has recently shown an increase from 0.14 to 0.21 per 100,000 population between 1983 and 2012 in the USA (Randle, et al. 2017). The prevalence is ~2% of all thyroid malignancies, 0.4–1.4% of all thyroid nodules, and it is detected in ~0.14% thyroids of subjects submitted to autopsy (Lim, et al. 2017; Tuttle, et al. 2014; Valle and Kloos 2011). The clinical presentation is mainly in the fourth and fifth decades of life with a small, but statistically significant, increase in diagnosis at a mean age from 50 to 54 years (Randle et al. 2017). MTC accounts for 13.4% of the total deaths attributable to thyroid cancer (Modigliani, et al. 1998).

Approximately 35% of patients with MTC who present with a palpable thyroid nodule have cervical metastases, and ~13% have distant metastases (Kebebew, et al. 2005; Roman, et al. 2006). The reported 10-year disease-specific mortality rate for MTC varies from 13.5 to 38% (Girelli, et al. 1998; Kuo, et al. 2018). The tumor stage at the time of diagnosis and the possibility of a complete surgical resection mostly determine the likelihood of attaining a cure for MTC. The classical main prognostic factors are age, tumor size, local and distant metastases, somatic M918T mutations, calcitonin, and CEA doubling times (Meijer, et al. 2010).

MTC presents as sporadic (75–80%) or inherited tumors (20–25%). Hereditary MTC appears as part of the MEN 2 syndrome. MTC is extremely rare in children, making the probability of a hereditary form very high. Germ-line mutations of the *RET* (REarranged during Transfection) proto-oncogene cause hereditary cancer, whereas somatic *RET* mutations are frequently present in sporadic disease (Eng, et al. 1995; Mulligan 2018). *RET* encodes a transmembrane receptor, and point-activating *RET* mutations promote

continuous phosphorylation of a distinct set of tyrosine residues, triggering intracellular signaling pathways responsible for cell survival, differentiation, and proliferation (Figure 1).

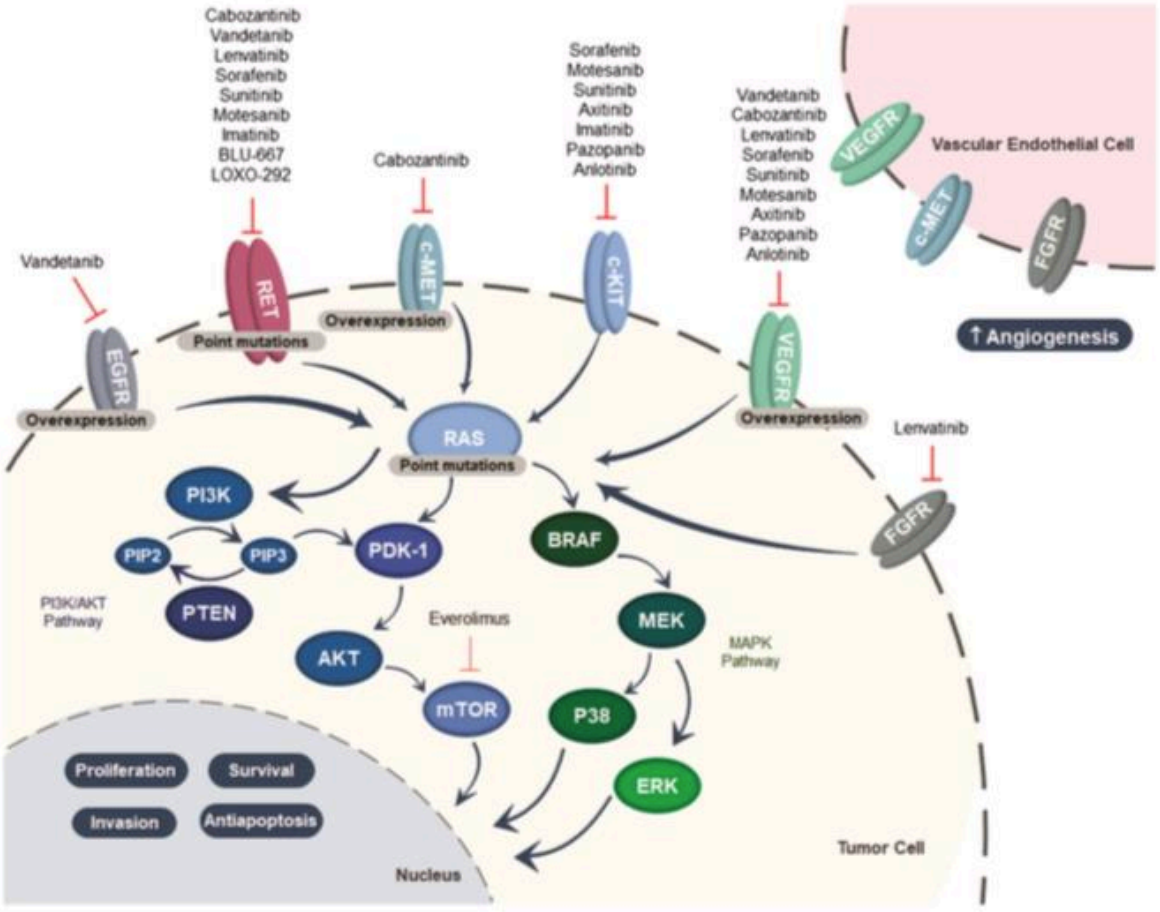

Figure 1. Illustration of the activated pathways and genetic aberrations involved in medullary thyroid cancer, as well as the molecular targeted-related compounds.

CLINICAL PRESENTATION AND GENOTYPE-RELATED PHENOTYPES

Hereditary MTC is usually associated with C-cell hyperplasia, multicentric, and bilateral (Schmid 2015), while the diagnosis of sporadic forms tends to be late, generally in the fifth or sixth decade of life (Heshmati, et al. 1997). Lymph node metastases occur in at least 35% of MTC patients at diagnosis, while distant metastases occur in approximately 20% of cases. A minority of patients with MTC present systemic manifestations that include diarrhea, flushing, or painful bone metastases (Elisei, et al. 2008; Hannah-Shmouni, et al. 2016; Kebebew, et al. 2000).

Hereditary MTC is inherited as an autosomal dominant syndrome that involves other endocrine neoplasias, with a variable degree of expressivity and an age-related penetrance, named multiple endocrine neoplasia type 2 (MEN 2). Interestingly, MEN 2 syndrome is classically defined by the occurrence of multicentric tumor formation in organs in which the *RET* proto-oncogene is expressed. This idea emerged because thyroid C cells have long been considered to be derived from neural crest cells. However, recent lineage-tracing experiments in mouse embryos have demonstrated that thyroid C-cell precursors are derived from anterior endoderm, specifically from the pharyngeal pouches from which the ultimobranchial bodies develop (Johansson, et al. 2015). These surprising results may disprove the current concept of a neural crest origin of thyroid C cells and would have implications for understanding coincidental tumorigenesis of MTC, pheochromocytoma (PHEO) and hyperparathyroidism (HPT), revealing new paths for investigation of the involved molecular mechanisms (Nilsson and Williams 2016).

The MEN 2 syndrome is classified according to the involved organs as multiple endocrine neoplasia type 2A (MEN 2A) and multiple endocrine neoplasia type 2B (MEN 2B) (Pelizzo, et al. 2007; Wells, et al. 2015). MEN 2A constitutes approximately 70–80% of all MEN 2 cases and classically includes four subtypes: Classical MEN 2A, MEN 2A associated with cutaneous lichen amyloidosis (CLA), MEN 2A and Hirschsprung's disease (HD), and familial medullary thyroid carcinoma (FMTC). Classical MEN 2A is characterized by the presence of MTC (95%), PHEO (30–50%) and HPT (10–20%). MEN 2A with CLA, a pruriginous lesion in the scapular region characterized by amyloid deposition, and MEN 2A with HD, caused by the absence of autonomic ganglia in the terminal hindgut that results in colonic dilatation, obstipation, and constipation are rare variants of the disease (Decker, et al. 1998; Gagel, et al. 1989).

Previously, considered a freestanding syndrome, FMTC, characterized by the presence of an inheritable MTC with no association with other endocrine neoplasia, is now regarded as a variant of the MEN 2A spectrum, in which the clinical presentation of MTC occurs later, and the prognosis is more favorable in comparison to the other forms of MTC. The reclassification into MEN 2A resulted from the concern that premature categorization of a family with FMTC could lead to a failure to identify a PHEO.

The clinical course of MTC in patients with MEN 2A is variable, and the disease progression is associated with codon-specific mutations in the *RET* proto-oncogene (Eng, et al. 1996a; Machens, et al. 2003). Approximately 98% of MEN 2A is associated with *RET* mutations in the cysteine-rich extracellular domain, particularly in exons 10 and 11, codons 609, 611, 618, 620, and 634, which is responsible for at least 85% of MEN 2A cases and correlated with the presence of PHEO, HPT, and CLA (Eng, et al. 1996b; Maciel, et al. 2019; Raue and Frank-Raue 2009; Scapineli, et al. 2016). Amino acid change in the intracellular domain of *RET* in exon 13 (codons 768,790 and 791), exon 14 (codons 804 and 844) and exon 15 (codon 891) are less frequent. Mutations in exon 8 (codon 533) is rare, but they have been described in a large Brazilian family (Da Silva, et al. 2003) and are most prevalent in familial cases in the Greek population (Maciel et al. 2019; Sarika, et al. 2015). In 2-5% of cases of apparently hereditary MTC no *RET* mutations are found (Leboulleux, et al. 2004). Of note, whole exome sequencing has recently identifies a germline *MET* mutation in two siblings with hereditary wild-type *RET* MTC (Sponziello et al., 2018).

A distinct MTC biological behavior, characterized by reduced aggressiveness and an older mean age at diagnosis, has been described for MEN 2A associated with mutations in noncysteine codons comparatively to mutations in cysteine codons (Raue and Frank-Raue 2009), and mutations in codon 611 tend to give rise to slow tumor progression than mutations in codons 618 and 620 (Machens, et al. 2018). On the other hand, more advanced stage and increasing risk of metastases correlated with mutations in codon position (609→620) near the juxtamembrane domain (Frank-Raue, et al. 2011). Interestingly, specific nucleotide and amino acid exchanges seem to have an impact on tumor behavior and aggressiveness in patients harboring codon 634 mutations (Punales, et al. 2003). Of interest, a case of aggressive sporadic MTC with a somatic *RET* C634R mutation and germ-line synonymous C630C mutation was reported.

Expression analysis has shown increased levels of *RET* transcript in C630C/C634R compared with that observed in 7 MTCs harboring only C634 mutations, suggesting that synonymous mutations can contribute to cancer progression (Pecce, et al. 2018).

The MEN 2B accounts for approximately 5% of the cases of MEN 2 and is characterized by a single phenotype, which includes diffuse ganglioneuromatosis of the tongue, lips, eyes, and gastrointestinal tract, marfanoid habitus, and alacrimia. MEN 2B patients present with MTC (90%), PHEO (45%), ganglioneuromatosis (100%), and Marfanoid habitus (65%). MTC in the setting of MEN 2B develops earlier and has a more aggressive course compared with MTC in other MEN 2 subtypes (Brandi, et al. 2001; Makri, et al. 2018). A specific mutation in *RET* exon 16, M918T, is almost invariably associated with MEN 2B and usually presents MTC development a few years after birth. Other mutations rarely associated with MEN 2B have been reported at codon 883 of exon 15; however, MTC in A883F carriers seems to present a more indolent course in comparison to M918T carriers (Mathiesen, et al. 2017). Double *RET* mutations involving codons 804/806, 804/778 or 804/904 have also been described (Kasprzak, et al. 2001; Kihara, et al. 2014; Menko, et al. 2002). A recent large, multicenter study has shown that over 80% of the cases of MEN 2B are *de novo RET* mutations, implying that the majority of children will be diagnosed after the recommended age of thyroidectomy. These observations highlight the importance to educate pediatricians and other health care providers to recognize the early nonendocrine manifestations of the disease (Castinetti, et al. 2019).

Sporadic MTC

The molecular mechanisms involved in sporadic MTC have not yet been clarified. Approximately 23–66% of sporadic MTC presents the somatic *RET* M918T mutation. Also, mutations in codons 618, 603, 634, 768, 804, and 883 and partial deletion of the *RET* gene have been described in a few tumors (Dvorakova, et al. 2008; Elisei, et al. 2008; Romei, et al. 2016). However, the mutations are not uniform throughout the tumor, suggesting that sporadic MTC might have a polyclonal origin or that these mutations are secondary events of MTC tumorigenesis (Eng, et al. 1996b; Romei, et al. 2018).

In addition to gain-of-function *RET* mutations, several *RET* variants have been associated with an increased risk of development or progression of MTC (Ceolin, et al. 2012a; Ceolin, et al. 2012b). Nevertheless, the mechanism by which these variants modulate MTC pathogenesis remains unclear. The exchange of bases in the DNA molecule may create an alternative splicing site, leading to the synthesis of a truncated protein, erroneous ligand binding, micro-RNA binding, or a change in mRNA structure and stability as well as in the number of copies (Borrego, et al. 1999). It is also possible that this neutral variant is in Linkage disequilibrium (LD) with an as yet unknown functional variant. Indeed, it has been shown that the S836S polymorphism is in LD with the intronic IVS1-126G>T variant found to be overrepresented in a cohort of sporadic MTC patients (Fernandez, et al. 2006). LD between *RET* S836S and 3'untranslated region (UTR) variants has also been demonstrated. Of note, the R*ET* mRNA sequence carrying the S836S/3'UTR haplotype presents higher structural and thermodynamic stability, suggesting a functional involvement of the 3'UTR variant allele in the posttranscriptional control of *RET* (Ceolin, et al. 2016). Sporadic MTC patients present higher DNA methylation levels compared to those with the inherited form or control individuals, which might suggest an epigenetic contribution to MTC tumorigenesis (Ceolin, et al. 2018). Moreover, epigenetic-related gene profiling shows significant increases of histone methyltransferases genes, which are involved in transcriptional regulation of gene expression, in patients with aggressive MTC (Sponziello, et al. 2014).

DIAGNOSIS AND PROGNOSTIC MARKERS

The clinical presentation of MTC traditionally consists of a palpable thyroid nodule, which may be solitary or appears in the context of a multinodular goiter. Subsequently, the diagnosis is performed through the typical diagnostic work-up of thyroid nodules (Haugen, et al. 2016). The routine use of serum calcitonin in the evaluation of thyroid nodules is not a consensus; the European Thyroid Society recommends it, but not the American Thyroid Association or Brazilian Society of Endocrinology (Maia, et al. 2014; Schlumberger, et al. 2012; Wells et al. 2015). There was an agreement that, in certain situations, such as

patients considered for less than total thyroidectomy or with suspicious cytology not consistent with papillary thyroid cancer, serum calcitonin measurement should be considered; In these situations, serum calcitonin presents a positive predictive value of 100% if > 100 pg/mL and 5% if between 10 and 100 pg/mL (Tormey, et al. 2017; Turk, et al. 2017; Wells et al. 2015). Besides, calcitonin measurement in the fine-needle aspiration (FNA) washout might be an additional tool when FNA biopsy findings are inconclusive or undetermined (Trimboli, et al. 2014). Nevertheless, one should keep in mind that false positive results have been reported in selected cases (Massaro, et al. 2009; Trimboli, et al. 2012).

Calcitonin is the most important MTC marker, as it is useful for diagnosis, surgical planning, follow-up, and prognosis. When compared to FNA biopsy for MTC diagnosis, calcitonin presents higher sensibility (nearly 100%) and specificity (95%) (Bugalho, et al. 2005). High levels of calcitonin may also occur in other medical conditions such as chronic kidney failure, hyperparathyroidism, neuroendocrine neoplasms, lung and prostate tumors and autoimmune thyroiditis (Karanikas, et al. 2004; Rosario and Calsolari 2016). Preoperative basal serum calcitonin correlates with the tumor size and extent of lymph node metastasis. Levels higher than 20, 50, 200, and 500 pg/mL were associated, respectively, with metastases to lymph nodes in the ipsilateral central and ipsilateral lateral neck, the contralateral central neck, the contralateral lateral neck, and the upper mediastinum. A biochemical cure is very unlikely in patients with preoperative serum calcitonin levels higher than 1000 pg/mL (Machens and Dralle 2010; Wells et al. 2015).

The diagnosis of hereditary MTC usually occurs in advanced stages on index cases, taking into account the development at early ages and the asymptomatic nature of the disease in the initial stages. However, the diagnosis is made in early stages or even in a premalignant phase in family members, due to the broad recommendation of genetic screening in all MEN 2 patients. Indeed, the molecular test of proband's relatives is of paramount importance since the earlier diagnosis and treatment increase the likelihood of cure of MTC (Punales, et al. 2008; Skinner, et al. 2005). Depending on the *RET* mutation, the MTC risk is classified as highest (M918T), high (C634F/G/R/S/W/Y and A883F) or moderate (all others), changing the time for initiating calcitonin level measurements and prophylactic thyroidectomy. Biochemical calcitonin monitoring might demarcate a "window of opportunity" for pre-emptive thyroidectomy without

central node dissection. For individuals harboring highest risk mutations, thyroidectomy should be performed early in life. For carriers of high-risk mutations, the thyroid surgery should be recommended before 5 years of age whereas those carrying moderate risk mutations might be followed every 6-12 months until serum calcitonin levels became elevated (Wells et al. 2015). Of interest, recent studies indicate that some mutations, classified as moderate risk by the ATA (codons 768, 790, 804), have a more indolent clinical course with a five-year-long expectant observation period under the premise that calcitonin levels remain within reference limits (Machens et al. 2018; Wells et al. 2015). Moreover, in a series of MEN 2A gene-carrier patients followed in a referral center in Italy, basal calcitonin levels below 60 pg/ml were always associated to an intrathyroidal MTC (Elisei, et al. 2012). These observations might suggest that the ideal timing for prophylactic thyroid surgery could be individualized, taking into account patient age, type of mutation, biomarkers and imaging exams. Stimulation calcitonin tests might be useful in the decision-making process regarding prophylactic surgery (Elisei, et al. 2012; Jarzab, et al. 2013). Nevertheless, although extensively used in the past, recent studies found a similar accuracy between basal and stimulated calcitonin levels, indicating that the new serum calcitonin assays with improved functional sensitivity decrease the significance of stimulation tests (Elisei, et al. 2012; Mian, et al. 2014).

THERAPEUTIC STRATEGIES

Surgery is the only curative treatment for MTC. Total thyroidectomy with central lymph node dissection is the procedure of choice and, depending on the serum calcitonin levels, and pre-operative cervical US imaging, a more extensive surgery with lateral neck dissection should be considered (Maia et al. 2014; Wells 2018; Wells et al. 2015). Patients with intrathyroidal tumor have a 10-year survival rate of 95.6%, whereas patients with regional stage disease or distant metastasis at diagnosis present overall survival rates of 75.5% and 40%, respectively (Roman et al. 2006). Interestingly, the absolute number of lymph node metstases seems to impact on the chances of biochemical cure after additional surgical

intervention. Scollo et al., (2003) have shown higher rates of calcitonin normalization in patients with less than 10 metastatic lymph nodes, as compared with those present a large number (57 vs. 4%) (Scollo, et al. 2003). Recently, Sosa J. and colleagues (2017) proposed a more accurate TNM risk stratification and potential treatment selection, lowering the risk of overtreatment for patients with stage I MTC. Based on the proposed new TNM grouping, the 5-year overall survival was 94% for stage I, 86% for stage II, 69% for stage III and 35% for stage IV (Sosa, et al. 2017). Patients with persistent or recurrent MTC localized to the neck are candidates for repeat neck operations. However, in the presence of widespread regional or metastatic disease, extensive surgery is not associated with a higher cure rate, and less aggressive procedures should be considered (Randle et al. 2017; Wells 2018). Patients with incidental, sporadic MTC and no evidence of residual disease might be safely observed after less extensive resections (Randle et al. 2017).

The presence and volume of residual disease should be assessed through calcitonin measurements to define the appropriate treatment and follow-up strategy after thyroid surgery. The ATA guidelines recommend investigation for distant metastases if serum calcitonin levels are above 500 pg/mL preoperatively or 150 pg/mL post-total thyroidectomy. Neck and chest CT, liver MRI, bone scintigraphy, and eventually [18F]-fluorodeoxyglucose (18F-FDG) PET-CT might be used to investigate distant metastases. The sensitivities of these tests for detecting metastatic disease vary from 50-80%, with a lower likelihood of identifying metastatic disease in those individuals with discrete calcitonin elevation (Giraudet, et al. 2007; Wells et al. 2015). Of interest, a recent study, which evaluated the performance of 68Ga PET-CT in detecting MTC lesions, indicates that it is highly sensitive in identifying bone lesions and could be a substitute for a bone scan and MRI (Castroneves, et al. 2018).

In the postoperative period, calcitonin and CEA may require weeks to reach their lowest levels, so the measurement should be performed at least 2 to 3 months after surgery. Since serum calcitonin and CEA levels may either persist steadily high for years or rapidly increases, the calculations of their doubling times (DT; available at the ATA website http://www.thyroid.org/thyroid-physicians-professionals/calculators/thyroid-cancer-carcinoma/) are more accurate to evaluate the disease progression. The 5-year and 10-year survival rates are 25 and 8%, respectively, when the doubling time is less than 6

months, and 92 and 37%, respectively, when the doubling time ranges from 6 months to 2 years. The calcitonin doubling time correlates with the survival and tumor recurrence rates, providing a better predictor of survival, whereas the CEA doubling time seems to be more useful for predicting prognosis (Meijer et al. 2010).

Recently, it has been shown that dynamic risk stratification is an excellent and useful tool to acquire prognostic information and can be used to modify the initial risk estimates by the classical TNM staging. The 5-year and 10-year recurrence rates vary from less than 1 to 8.5% in patients who achieve an excellent response, defined as an undetectable calcitonin level after surgery. Furthermore, the nomenclature excellent, biochemical incomplete and structural incomplete response, which has been successfully used to characterize the response to therapy and predict the clinical outcome in differentiated thyroid cancer, has also been shown to be useful in MTC (Choi, et al. 2018; Kwon, et al. 2016; Lindsey, et al. 2015).

Other potential prognostic markers have been studied in recent years. Classically used as a marker for pancreatic neoplasms, higher levels of carbohydrate antigen (CA19.9) have been reported in patients with very aggressive MTC disease, low calcitonin levels, and increased CEA levels (Elisei, et al. 2013a; Milman, et al. 2011). Based on an evaluation of serum CA19.9 levels in patients with advanced structural recurrent/persistent MTC, an elevated serum CA 19.9 value appears to be a predictive factor for poor prognosis and identifies those cases with a higher risk of short-term mortality (Elisei, et al. 2015). CA19.9 has also been shown to be associated with an advanced disease stage in a small pilot study (Milman, et al. 2015). However, in a study conducted by our group, immunohistochemical analysis of CA19.9 was not associated with age, sex, calcitonin, CEA, or local or distant metastases (CVF Vargas, L Ceolin, AF Benine, MS Graudenz & AL Maia, unpublished observations).

GENERAL THERAPEUTIC APPROACH IN METASTATIC MEDULLARY THYROID CARCINOMA

When evaluating a patient with advanced MTC, the following questions should be considered during decision-making: Is the patient symptomatic or asymptomatic? Is the loco-regional disease

controlled? Where are the metastases located? Are there lesions that require intervention due to imminent risk or associated symptoms? What is the speed of metastatic disease progression? Unfortunately, it is not always possible to get a definitive answer for some of these questions.

For patients with locally advanced disease that is not amenable to surgery or those who present distant metastasis, there is no effective therapeutic, curative option. Chemotherapy and external beam radiation therapy for the metastatic cervical recurrent disease have limited response rates (Brierley and Tsang 1996; Nocera, et al. 2000). The response rate to cytotoxic chemotherapy seems to be approximately 20%, with most studies performed on limited numbers of patients and without robust evaluation criteria such as RECIST (Hadoux and Schlumberger 2017). External beam radiation therapy (EBRT) may be recommended to improve locoregional control in patients at high risk of cervical relapse. Few studies have shown an improvement in locoregional control but no survival benefit, confirming that although neck disease can be controlled in high-risk patients, the progression of distant disease and subsequent death are still a significant problem (Brierley and Sherman 2012; Call, et al. 2013; Martinez, et al. 2010; Schwartz, et al. 2008).

Upon planning the therapeutic strategy, it is essential to keep in mind that some patients with metastatic MTC present indolent disease and good long-term prognosis, whereas others develop a progressive disease that requires close evaluation for immediate treatment. The schematic flowchart (Figure 2) summarizes a therapeutic strategy to metastatic MTC. Expectant management can be appropriated for asymptomatic individuals with indolent, low burden disease whereas urgent therapy might be indicated in the presence of lesions which are associated with a high risk of serious complications, such as large brain metastases, a spinal cord compression or a lesion growing into an airway, or bone metastases with an imminent risk of fracture. Embolization or cryoablation may be beneficial in selected cases to decrease the tumor burden, pain, or refractory diarrhea associated with liver metastases (Fromigue, et al. 2006). Localized EBRT and/or bisphosphonates administration should be considered for painful bone metastases or prevention of skeletal-related events (Beuselinck, et al. 2012; Farooki, et al. 2012).

Patients with advanced MTC may experience paraneoplastic debilitating diarrhea due to hypersecretion of calcitonin, VIP (Cox, et al. 1979), or increases on intestinal motility (Rambaud, et al. 1988). Antimotility agents (loperamide or codeine) may be initially used as first-line therapy. Further options for unsuccessful cases include somatostatin analogs (Mahler, et al. 1990; Zatelli, et al. 2006). For those patients with extensive liver metastases, surgical resection, percutaneous radiofrequency ablation, or arterial chemoembolization of might be considered in an attempt to reduce the calcitonin levels (Fromigue et al. 2006). The most common ectopic hormones, CRH or ACTH, can rarely cause paraneoplastic Cushing's syndrome (0.7% cases), accounting for up to 2-6% of ectopic Cushing's syndrome cases (Barbosa, et al. 2005). Until recently, the management of this challenging situation, associated extreme morbidity and mortality, was limited to surgical removal of metastatic disease, medical therapy with anti-adrenal compounds or bilateral adrenalectomy. Nevertheless, recent reports indicate successful treatment of MTC-related Cushing syndrome with TKIs (Barroso-Sousa, et al. 2014; Nella, et al. 2014).

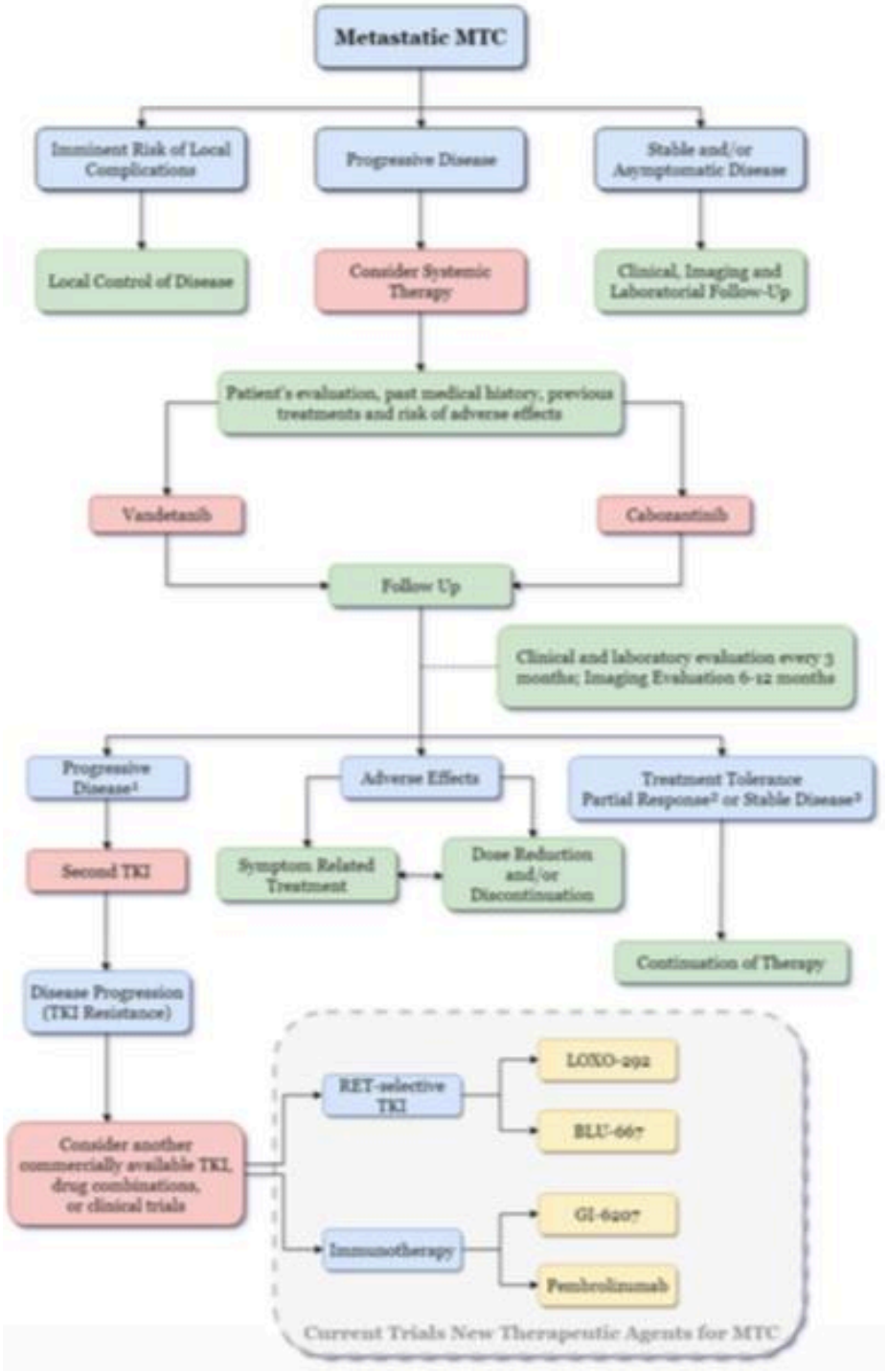

Figure 2. Schematic flowchart for the management of patients with metastatic MTC.

SYSTEMIC THERAPY FOR ADVANCED MEDULLARY THYROID CARCINOMA

General overview

Cumulative knowledge regarding the distinct signaling pathways and multiple genetic abnormalities involved in the pathogenesis of cancer has allowed the development of targeted molecular therapies. Protein kinases are characterized by their ability to catalyze the phosphorylation of tyrosine amino acid residues in proteins, activating the various intracellular signaling pathways, cell proliferation, differentiation, migration, and anti-apoptosis. Therefore, tyrosine kinase inhibitors (TKIs) may provide a therapeutic benefit in cancer by blocking tyrosine kinase-dependent oncogenic pathways. TKIs can be specific to inhibit one or several tyrosine-kinase receptors (multikinase inhibitors, MKIs), (Broekman, et al. 2011; Lemmon and Schlessinger 2010).

SIGNALING PATHWAYS IMPLICATED IN MEDULLARY THYROID CANCER

Uncontrolled tyrosine kinase receptor activation is one of the primary mechanisms of cancer development and progression. In normal thyroid C cells, signaling pathways such as RET, RAS/MAPK, PI3K, c-MET, and mTOR modulate a wide range of intracellular processes, including cell proliferation, differentiation, migration, and apoptosis. Diverse molecular-driven alterations in these signaling pathways are involved in thyroid carcinogenesis (Mulligan 2014; Santarpia, et al. 2010). The role of RET tyrosine kinase receptor in MTC pathogenesis has been well documented. Vascular endothelial growth factor (VEGF) and hepatocyte growth factor (c-MET), as well as their tyrosine kinase receptors, are overexpressed in MTC and play critical roles in pathogenesis, progression, and disease recurrence (Capp, et al. 2010; Papotti, et al. 2000).

RET Pathway

Hereditary MTC is caused by gain-of-function mutations of the RET receptor that lead to constitutive RET kinase activity. In this oncogenic mechanism, MEN 2A-related mutations activate RET by inducing disulfide-linked homodimerization, in which a cysteine residue is mutated to a noncysteine residue, and a partner cysteine that is involved in the formation of a disulfide bond become free and form an aberrant intermolecular disulfide bond between two mutants RET. In MEN 2B mutations, which occur in the tyrosine kinase 2 domain, RET is activated in monomeric form, probably due to a conformational change in the catalytic core of the kinase domain. These mutations increased ATP-binding and kinase activity, allowing robust activation of downstream signals (Mulligan 2018).

The *RET* gene was identified in 1985 by Takahashi and cols, mapping on 10q11.2 and containing 21 exons spanning a region of 55,000 bp (Takahashi, et al. 1985). RET is a member of the cadherin superfamily and encodes a tyrosine kinase receptor, which is a cell-surface molecule that transduces signals for cell growth, proliferation, differentiation, migration, survival, and apoptosis. RET proteins are derived from a single polypeptide core of 120 kDa and modified to 150 kDa and 170 kDa by post-translational glycosylation. Only the fully mature glycosylated 170-kDa RET protein isoform is present on the cell surface, whereas the immature 150-kDa isoform is confined to the Golgi (Takahashi, et al. 1993; Takahashi, et al. 1991). Alternative splicing of the RET gene results in the RET51, RET43 and RET9 isoforms, which differ at their carboxyl termini (Carter, et al. 2001; Myers, et al. 1995; Tahira, et al. 1990).

The RET receptor comprises three domains: an extracellular, a transmembrane, and an intracellular domain. The extracellular domain includes regions that are homologous to the cadherin family of cell adhesion (cadherin-like) molecules that induce and stabilize conformational changes needed for interactions with the ligands and coreceptors and a large region enriched in cysteine residues (cysteine-rich region), which is responsible for the tertiary structure and formation of dimers. The intracellular domain contains two tyrosine-kinase domains (TK1 and TK2) that are separated by 28 amino acids. These subdomains contain the tyrosine residues are phosphorylated during receptor activation and are involved in the activation

of the signaling pathways (Takahashi, et al. 1988). The tyrosine residues 905, 1015, 1062 are conserved in all three RET isoforms, but the tyrosine residue 1096 is present only in the long (RET 51) isoform.

RET is a receptor tyrosine kinase essential for the normal development and maturation of different tissues. Under normal conditions, RET is activated by a group of proteins of the glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs), including GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN). RET does not directly bind to GFLs, requiring an additional coreceptor, a GDNF family receptor-α (GFRα). The GFL-GFRα complex binds to RET, inducing RET dimerization and a subsequent autophosphorylation on multiple tyrosine residues within the intracellular tyrosine kinase domain (Airaksinen and Saarma 2002).

Studies using transgenic mouse models have demonstrated that *Ret* oncogenes can drive MTC development. Mice expressing *Ret*-C634R or *Ret*-M918T under the control of the calcitonin gene promoter developed MTC (Acton, et al. 2000; Michiels, et al. 1997). Additionally, transgenic mice carrying *Ret*-C634R under the control of a ubiquitous viral promoter developed MTC, suggesting that murine C-cells are highly susceptible to RET-mediated transformation (Kawai, et al. 2000). However, knock-in of the M918T mutation into the mouse endogenous *Ret* gene caused CCH but not MTC, suggesting that, in the background of a normally expressed *Ret*-mutant allele, the accumulation of secondary genetic alterations is required for the development of MTC (Smith-Hicks, et al. 2000).

Interestingly, RET protein has been shown to induce cell death in the absence of their ligands (GFL-GFRα), while in the presence of their ligands a completely different signal is transduced. In the absence of ligand, RET exerts pro-apoptotic activity, and the addition of GDNF is then sufficient to block RET apoptotic activity. This finding implies that a single mutation may simultaneously induce increased mitogenic signaling and reduce pro-apoptotic activity (Bordeaux, et al. 2000).

RAS Pathway

RAS gene mutations have been found in 0-68% *RET*-negative MTC (Ciampi, et al. 2013; Moura, et al. 2015; Moura, et al. 2011), and a recent meta-analysis has shown that the RAS mutation appears to lack significant prognostic value in predicting tumor aggressiveness (Vuong, et al. 2018). In our center, we identified a mutation in exon 2 of H-*RAS* in 3.8% of patients with sporadic MTC, 70% of whom were positive for somatic M918T in *RET* (CV Ferreira & AL Maia, unpublished observations). The oncogenic *RAS* mutations and mutations in other components of the RAS/MAPK signaling pathway appear to be mutually exclusive events in most tumors, indicating that the deregulation of Ras-dependent signaling is an essential requirement for tumorigenesis (Ciampi et al. 2013; Moura et al. 2011; Nikiforova, et al. 2013).

RAS genes (*H-RAS*, chromosome 11; *K-RAS*, chromosome 12; and *N-RAS*, chromosome 1) encode highly related G-proteins that play a central role in intracellular signal transduction by activation of the MAPK and other signaling pathways, such as PI3K/AKT (Fernandez-Medarde and Santos 2011; Santarpia et al. 2010). The *H-RAS, K-RAS*, and *N-RAS* genes all have a similar exonic structure, and therefore all probably derive from a common, spliced ancestral gene (Shimizu, et al. 1983).

The molecular mechanism proposed for RAS-derived tumorigenesis is the constitutive activation of distinct pathways that are linked to the functional control of a vast assortment of cellular outcomes, including cell cycle progression, growth, migration, cytoskeletal changes, apoptosis, and senescence (Fernandez-Medarde and Santos 2011; Santarpia et al. 2010). The ras-mutated protein mediates its effects on cellular proliferation in part by activation of a cascade of kinases: RAF (A-RAF B-RAF and C-RAF), dual-specificity mitogen-activated protein kinases (MEK1/2), extracellular signal-regulated kinases (ERK1/2) and p38 mitogen-activated protein kinase. RAS also activates the PI3K pathway via direct interaction with the catalytic subunit of the protein. PI3K activation leads to the accumulation of the 2nd messenger, phosphatidylinositol 3,4,5-trisphosphate (PIP3), resulting in pyruvate dehydrogenase kinase isozyme 1 (PDK1) and v-akt murine thymoma viral oncogene homolog (AKT) activation (Krasilnikov 2000; Vojtek and Der 1998).

MET Pathway

Overexpression of MET and co-expression of HGF-MET has been reported in MTC tumors and has been associated with multifocality (Papotti, et al. 2000; Ricarte-Filho, et al. 2009). Silencing of the *MET* proto-oncogene has resulted in impairment of the execution of the fully invasive growth program in vitro, lack of tumor growth and decreased generation of experimental metastases in vivo (Corso, et al. 2008). Crosstalk has been demonstrated between MET and RET at transcriptional and signaling levels, leading to the promotion of thyroid cell transformation and invasive phenotypes (Bentzien, et al. 2013; Cassinelli, et al. 2009).

The *MET* proto-oncogene is located on chromosome 7q21-31 and encodes a single-pass tyrosine kinase protein. MET kinase is a cell surface receptor for hepatocyte growth factor (HGF), a cytokine that conveys a unique combination of pro-migratory, anti-apoptotic, and mitogenic signals expressed in the epithelial cells of many organs during embryogenesis and in adulthood (liver, pancreas, prostate, kidney, muscle, and bone marrow) (Cooper and Spaulding 1984; Gonzatti-Haces, et al. 1986; Park, et al. 1986). In tumor cells, MET activation triggers a diverse series of signaling cascades resulting in cell growth, proliferation, invasion, and protection against apoptosis (Birchmeier, et al. 2003; Liu, et al. 2008). Signaling for growth and mitogenesis occurs via the RAS-MAPK signaling pathway and plays an essential role in the epithelial-to-mesenchymal transition that results from loss of intracellular adhesion via cadherins and integrins, with a change in cell shape (Boccaccio and Comoglio 2006).

mTOR pathway

The oncogenic RET activity in MTC seems to be partially modulated by overactivation of the PI3K/Akt/mTOR pathway (Drosten, et al. 2004). Interestingly, studies have shown that mTOR has a higher activation in lymph node metastases than in primary MTC and that the expression of eiF4E has a strong correlation with the tumor stage, suggesting a role of mTOR in tumor progression (Kouvaraki, et al. 2011;

Tamburrino, et al. 2012). Besides, mTOR activation appears to be an early event in C-cell transformation, playing a role early in the MTC tumorigenic process (Tamburrino et al. 2012).

The *mTOR* gene is located on chromosome 1p36.22 and contains 60 exons. *mTOR* encodes a serine/threonine kinase, in the family of phosphatidylinositol kinase-related kinases, which is involved in the regulation of cell proliferation, apoptosis, the cell cycle, angiogenesis, metabolism, and protein synthesis (Meric-Bernstam and Gonzalez-Angulo 2009). mTOR functions as part of 2 structurally and functionally distinct signaling complexes: mTOR complex 1 (mTORC1), which consists of mTOR, mammalian LST8 (mLST8), proline-rich Akt substrate 40 (PRAS40), and raptor; mTOR complex 2 (mTORC2), which includes of mTOR, mLST8 (GßL), mSIN1, PRR5 (protor), and Rictor (Jacinto, et al. 2006; Martin, et al. 2008; Wullschleger, et al. 2006).

The deregulation of mTOR pathway activation is observed in several types of cancer. The main pathway of mTOR activation is PI3K / Akt. Specific growth factors are responsible for stimulating RTKs that lead to PI3K / Akt activation. Once stimulated these receptors PI3K is recruited and catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3) and thus activates Akt. The control of Akt activation and, consequently of mTOR, is done by PTEN, a tumor suppressor that converts PIP3 to PIP2 thus inhibiting the activation of Akt (Meric-Bernstam and Gonzalez-Angulo 2009; Sekulic, et al. 2000).

MOLECULAR TARGET THERAPY: MULTIKINASE INHIBITORS

The advances in knowledge of the molecular mechanisms and intracellular signaling pathways involved in MTC pathogenesis have allowed the development of target therapy, promoting noteworthy developments and new perspectives on metastatic MTC therapy.

Several multikinase inhibitors (MKI) compounds have been tested for MTC treatment, including motesanib (Schlumberger, et al. 2009), sorafenib (de Castroneves, et al. 2016; Lam, et al. 2010), sunitinib (Carr, et al. 2010), axitinib (Cohen, et al. 2008), imatinib (de Groot, et al. 2007), pazopanib (Bible, et al.

2014), anlotinib (Sun, et al. 2018), lenvatinib (Schlumberger, et al. 2016), vandetanib (Wells, et al. 2012), and cabozantinib (Elisei, et al. 2013b) (Figure 1). Because patients with metastatic MTC may present indolent disease and long life expectancy, prospective trials use surrogates than overall survival (OS) to evaluate drug efficacy. The objective response rate (ORR) and the progression-free survival (PFS) are the most used outcomes since they show a better correlation with OS (Hadoux and Schlumberger 2017).

Vandetanib and cabozantinib are the only MKIs approved for advanced MTC treatment. The first approved compound, vandetanib, selectively targets RET, VEGF, and epidermal growth factor (EGF) receptors (Wedge, et al. 2002). The efficacy of vandetanib was evaluated in 331 individuals with metastatic MTC who were randomized to receive vandetanib (300 mg) or placebo (Wells et al. 2012). The results showed a significant increase in PFS in the vandetanib-treated group [30.2 vs. 19.2 months; HR=0.46, 95% CI=0.31–0.69]. Vandetanib has also been successfully used to treat children with MEN 2B (Fox, et al. 2013). The second approved compound, cabozantinib, is a c-MET, VEGFR2, and RET MKI. A randomized study of 330 individuals with documented MTC progression demonstrated a significant increase in PFS in the cabozantinib-treated group (11.2 vs. 4.0 months; HR=0.28, 95% CI=0.19–0.40, P<0.0001) (Elisei et al. 2013b). The effect of vandetanib or cabozantinib on the survival of MTC patients remains unknown, but interim analyses have not revealed any differences between the two drug-treated and placebo groups (Elisei et al. 2013b; Wells et al. 2012).

Lenvatinib, an MKI of the VEGFR-1, 2, and 3, FGFR-1–4, PDGFRa, RET, and KIT signaling networks, was evaluated in a phase 2 trial. Fifty-nine patients with unresectable progressive MTC were included in that study. The disease control rate was 80% (95% CI: 67–89%), which is the highest reported rate to date. Of interest, the objective response rate (ORR) was similar between patients with (35%) and without (36%) prior anti-VEGFR therapy, confirming the lack of cross-resistance among MKIs (Schlumberger et al. 2016).

Given that RET and RAS activate the PI3K/AKT/mTOR pathway, a small phase 2 trial was conducted to evaluate the efficacy of everolimus, an mTOR inhibitor approved for the treatment of neuroendocrine tumors and renal cell carcinoma, in patients with progressive metastatic or inoperable MTC

(Schneider, et al. 2015). Seven patients were enrolled, of whom five (71%) showed stable disease. The median PFS was 33 weeks, and none objective responses were observed. Similar findings were observed in another everolimus phase 2 trial that included 9 MTC patients (Lim, et al. 2013), indicating that everolimus alone has limited activity against MTC. Nevertheless, promising data have been reported from a phase 2 trial in patients with progressive, advanced thyroid cancer who received everolimus in combination with sorafenib (Sherman, et al. 2016). In another report, everolimus was prescribed in addition to vandetanib in a patient who presented disease progression and was observed a 25% tumor reduction (Heilmann, et al. 2016). The combination of RET- kinase inhibitors and mTOR inhibitors might be an exciting dual targeting strategy, but it awaits further evaluation in clinical trials.

A limitation of MKI therapy is that the tumor cells might develop an escape mechanism, allowing the tumor to start to grow again after a variable period of treatment. This phenomenon is independent of the type of MKI used or tumor treated (Arao, et al. 2011). Secondary mutations in the kinase domains that sterically block the binding of MKIs, usually downstream from the TKI target, or in parallel pathways that result in a mechanism to bypass the action of the drug, have been demonstrated in other tumors, but is still unclear in MTC (Liu, et al. 2018; Viola, et al. 2016). Interestingly, a suggestive case of an acquired *RET* V804M gatekeeper resistance mutation to vandetanib has been described (Subbiah, et al. 2018b). In such cases, a second MKI might be considered. Of note, the discontinuation of an MKI treatment could lead to a rapid increase in tumor growth and disease progression (Resteghini, et al. 2017; Trimboli, et al. 2018).

MUTATIONAL PROFILE AND RESPONSE TO MULTIKINASE INHIBITOR THERAPY

Several recent studies have indicated the potential clinical relevance of the identification of oncogenic driver alterations on molecular target therapeutic strategy. In the Phase III trial of vandetanib, patients with sporadic MTC harboring a somatic *RET* M918T mutation had a higher response rate to vandetanib as compared with patients without this mutation (54.5 vs. 32%), although the results were inconclusive due to the small sample size (Wells et al. 2012).

In the cabozantinib phase III trial, patients with *RET* mutation exhibited a longer PFS when compared with the placebo group (60 vs. 20 weeks), with the subgroup of patients harboring *RET* M918T achieving the greatest PFS (61 vs. 17 weeks). Patients with RAS mutations treated with cabozantinib also exhibited a longer PFS when compared with those treated with placebo (Sherman et al. 2016). Subsequent exploratory analyses have shown a statistically nonsignificant increase in the OS on the group who received cabozantinib as compared with placebo. Of note, the most significant benefits of cabozantinib treatment were observed in patients with RET M918T positive tumors (Schlumberger, et al. 2017). Despite these findings, there is no specific recommendation for treatment based on RET status. Some patients without documented RET mutations have benefited from MKI therapy. In vitro studies have shown that *RET* codon 804 and 806 mutations confer resistance to vandetanib therapy (Carlomagno, et al. 2009; Carlomagno, et al. 2004).

MTC is a highly vascularized tumor, and overexpression of VEGF and its co-receptors have already been shown in MTC samples (Capp et al. 2010). Angiogenesis is critical for tumor growth, and the MKI anti-angiogenic effect is likely to play a role in response to therapy. Of interest, angiogenesis appears to be more intense in RET positive tumors, a feature that might increase their susceptibility to antiangiogenic treatment (Verrienti, et al. 2016).

THE CHOICE OF THE FIRST-LINE DRUG

The chronic use and side-effect profiles of MKIs must be taken into account when selecting patients since it is not clear which patients will benefit the most from TKI therapy. The criteria for initiating therapy include a high tumor burden and a rapid rate of disease progression, tumor involvement that threatens vital structures that cannot be managed by localized therapy. Only a selected group of patients with metastatic MTC should be considered for systemic therapy (Figure3).

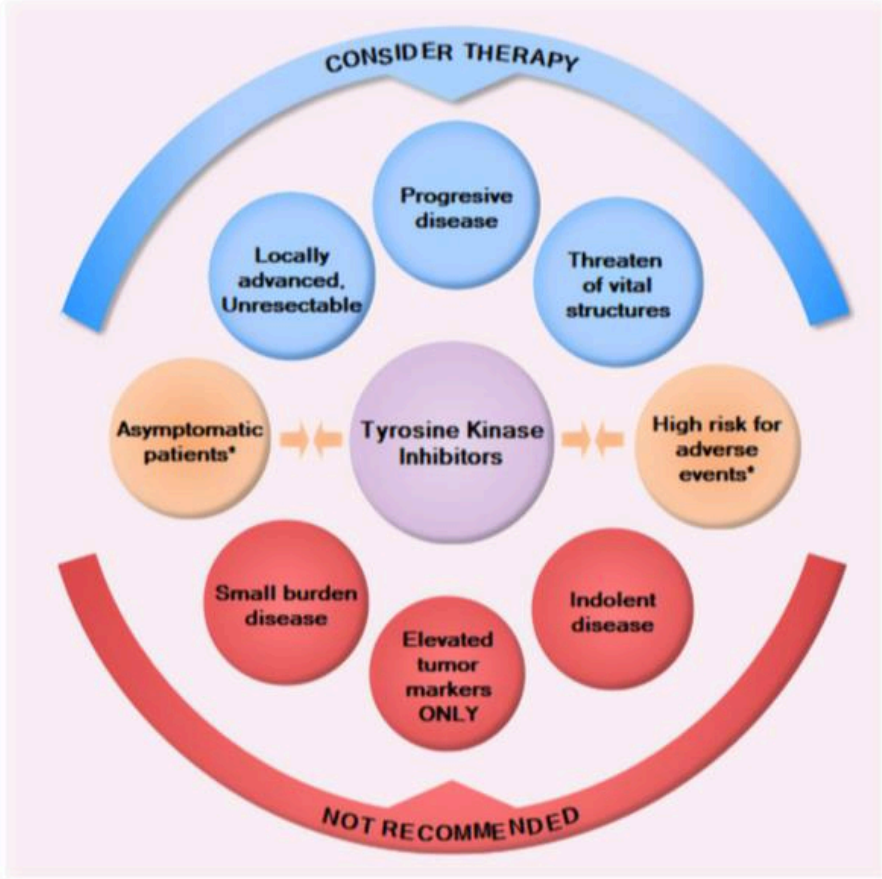

Figure 3. Schematic diagram of the main aspects of disease presentation to be considered before recommend or not MKI therapy in patients with metastatic MTC.

Despite the established benefits of MKI for PFS, it is essential to consider the several adverse effects often noticed during their use and how much they can impact the patients' life quality. The majority of MKI-related adverse events are familiar among the different drugs. The most frequent adverse events are diarrhea, rash, fatigue, and nausea. The most common AEs are usually of mild intensity (grade 1 or 2) and can be easily prevented or managed with symptom-related treatment, but in a non-negligible percentage of cases, dose reduction (up to 79% for cabozantinib and 35% for vandetanib) was needed in clinical trials (Elisei et al. 2013b; Viola et al. 2016). MKI-induced hypothyroidism is also frequent and requires an increase in the levothyroxine dose. Adverse events might be severe or life-threatening (G3–G4) in 5–10% of cases. MKI-related grade 5 adverse events have also been reported (de Groot, et al. 2007; Elisei, et al. 2013b; Lam, et

al. 2010; Scheffel, et al. 2013; Schlumberger et al. 2016; Schlumberger et al. 2009; Wells et al. 2012). Of interest, recent studies examining the use of vandetanib and sorafenib outside of a clinical trial have reported similar adverse event profiles (Chougnet, et al. 2015; de Castroneves et al. 2016).

In addition to the different side effect profiles of the MKIs, the attending physician must also take into account the patient's risk factors, past medical history, and adverse effect tolerance (Maia, et al. 2017). Particular caution should be taken when prescribing MKIs for patients with a medical history of hemoptysis and hemorrhages, tumor invading vital structures of the neck, radiation treatment of the neck or mediastinum since they may be at higher risk for hemorrhages and fistula formation, a rare but life-threatening antiangiogenic MKI adverse event (Blevins, et al. 2014). Vandetanib carries a higher risk for prolongation of the QT interval and should be avoided in patients with heart conduction disorders (Cabanillas, et al. 2014; Massicotte, et al. 2013). The use of vandetanib would be a better choice for patients whose occupation requires the use of the hands (e.g., carpenters, musicians) since the hand-foot syndrome is a common side effect of cabozantinib (Bastholt, et al. 2016). The management of side-effects related to MKI is essential to maximize clinical benefits and increase the patient's quality of life (Bastholt, et al. 2016; Grande, et al. 2013).

SELECTIVE TYROSINE KINASE INHIBITORS

Despite the advances in the management of metastatic MTC in the last decade, the clinical experience with the MKIs has been somewhat disappointing. While MKIs have increased the PFS, there was no improvement in OS. Virtually, all studies have shown a relatively low rate of partial responses, absence of complete response, and eventual tumor progression due to drug resistance. These drawbacks of MKI therapy may be partially explained by 'off-target' side-effects that limit the drug doses and consequently the degree of RET-specific inhibition. The vast and common adverse effects of MKIs results from concurrent inhibition of other targets, such as VEGFR2/KDR and lead to dose reductions or discontinuation (Romei et. al., 2016, Wells et. al., 2018). Recently, new next-generation small-molecule

TKIs designed for highly potent and selective targeting of oncogenic RET alterations have been developed with the goal of promote a potent RET pathway inhibition and improve the pharmacokinetic properties of the currently available MKIs (Subbiah et. al., 2018).

LOXO-292 and BLU-667

LOXO-292 is an orally bioavailable compound, selective and highly active RET inhibitor in preclinical models of RET-altered cancers *in vitro* and *in vivo*. In contrast to MKIs, LOXO-292 retains nanomolar potency against various RET alterations, with potential antineoplastic activity. A phase 1 study was designed to evaluate the safety, tolerability, pharmacokinetics and preliminary anti-tumor activity of LOXO-292 in patients with advanced solid tumors. The study included 82 patients, including 29 RET-mutant MTC tumors. The ORR in MTC patients was 45% (CI 95%: 24-68%). Tumor reduction was achieved in (9/20) 49% of MTC tumors, including a patient with a *RET* V804M mutation, and MKI pretreated patients. Ninety percent (19/24) of the MTCs had a ≥50% decrease in serum calcitonin. Overall, the compound appeared to be well tolerated among the patients. AEs (≥10%) were fatigue (20%), diarrhea (16%), constipation (15%), dry mouth (12%), nausea (12%) and dyspnea (11%). Only two treatment-related AEs ≥grade 3 were reported: tumor lysis syndrome (DLT) and increased ALT. Most MTC patients (93%; 27/29) remained on treatment (Subbiah et al. 2018b). ClinicalTrials.gov Identifier: NCT03157128.

BLU-667 is also a highly selective RET inhibitor. In vitro, BLU-667 demonstrated ≥10-fold increased potency over approved MKIs against oncogenic *RET* variants and resistance mutants. In vivo, BLU-667 potently inhibited the growth of thyroid cancer xenografts driven by various *RET* mutations and fusions without VEGFR-2 inhibition. To investigate the clinical impact of BLU-667, a phase I, first-in-human, the dose-escalation study was conducted. Fifty-one patients were enrolled with unresectable advanced solid tumors. Of them, 29 patients were found to have *RET*-mutant MTC. Overall, BLU-667 appeared to be well tolerated among the patients. The most common AE was grade1 constipation (23%). Grades 3 to 4 AEs were also found, including hypertension (8%) and neutropenia (4%). Additional AEs

included fatigue, diarrhea and a decrease in white blood cells (2% each). There were no reports of grades 4/5 AEs (Subbiah, et al. 2018a). Of note, the closest BLU-667 kinase off-target identified was Janus kinase 1 (JAK1). The side effects of JAK inhibition (reduced reticulocytes, red blood cells, neutrophils/monocytes) has not observed among the patients tested, suggesting the preferential activity of BLU-667 for RET versus JAK. ClinicalTrials.gov Identifier: NCT03037385.

IMMUNOTHERAPY

In the last few years, immunotherapy has transitioned from a promising to a well-established option as an oncological treatment for several types of malignancies, acting as an immune checkpoint inhibitor (Emens, et al. 2017). Preclinical studies on MTC have revealed potential new treatments through the use of immunotherapy (Naoum, et al. 2018). Several ongoing trials are investigating this type of therapy, including a phase II trial studying a therapy directed toward cells presenting CEA (GI-6207), a therapy focused on programmed death ligand 1 (PDL1) with the use of pembrolizumab (Arasanz, et al. 2017). Despite the lack of published results regarding the efficacy of these compounds on advanced MTC, all of these drugs have the potential to serve as new treatments.

CONCLUSION AND PERSPECTIVES

MTC is a very rare cancer with a good prognosis when diagnosed at early stages. For patients with advanced or metastatic disease, there is no effective therapeutic, curative option. In recent years, MKI therapy led to increases in the PFS, but no changes in the OS has been demonstrated to date. These compounds commonly cause toxicity, and it is crucial to establish an appropriate stratification of the clinical risk of patients to whom these drugs will be administered. The relatively low rate of partial responses and eventual tumor progression indicate the need to synergistic combinations of therapeutic targets whereas significant off-target toxicities may occur, limiting the degree of TKI-specific inhibition. Recently, next-

generation small-molecule TKIs designed for highly potent and selective targeting of oncogenic RET alterations have been developed, and with the emergence of immunotherapy as an effective cancer treatment, there is hope for new promising drugs for the treatment of advanced MTC.

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Conflicts of interest

A.L.M. has served as an advisor/speaker for Sanofi-Genzyme within the past 2 years. A.L.M. and C.V.F.V. have served as principal investigator and coordinator, respectively, in multicenter studies for Astra-Zeneca and Sanofi-Genzyme within the past 2 years. L.C., A.F.B., and M.A.S.D. have no conflicts of interest.

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Parte II

UNDETECTABLE POSTOPERATIVE CALCITONIN IS A STRONG PREDICTOR OF LONG-TERM DISEASE-FREE SURVIVAL IN MEDULLARY THYROID CARCINOMA

Undetectable postoperative calcitonin is a strong predictor of long-term disease-free survival in medullary thyroid carcinoma

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Abstract

Background: Calcitonin is widely used in the diagnosis, prognosis, and follow-up of patients with medullary thyroid carcinoma (MTC). The prognostic value of undetectable postoperative calcitonin (POCal) in long-term disease outcomes is still uncertain.

Objective: To evaluate the role of POCal as a prognostic marker for disease-free survival in MTC.

Methods: We collected data from the medical records of patients with MTC attending 2 tertiary teaching hospitals. We divided patients according to POCal into 2 groups: undetectable (below the reference value) or detectable serum calcitonin. Final outcome was determined by disease status, defined as disease-free (undetectable calcitonin and no evidence of disease on imaging), persistent disease (detectable calcitonin with or without structural disease), or disease-related death.

Results: We included 453 patients with MTC in the study. Mean age at diagnosis was 41.7 (SD, 18.4) years; 60.6% were women, and 54.4% had sporadic MTC. Median tumor size was 2.0 (1.1–3.5) cm; 191 (45.4%) had lymph node metastasis and 78 (18.5%) had distant metastasis. At the first postoperative evaluation (3–6 months after surgery), 149 patients (42.9%) had undetectable POCal. Remarkably, after a median follow-up of 8.5 (2.0–13.8) years, 127 (90.1%) of them were disease-free, whereas 14 (9.9%) had persistent biochemical disease with stable calcitonin levels and no evidence of structural disease. No patient died in the undetectable POCal group. In the detectable POCal group, 19 (9.8%) progressed to disease-free status, 50 (25.9%) had biochemical disease, 61 (31.6%) had persistent structural disease, and 63 (32.6%) died of disease-related events.

Conclusion: POCal appears to be a strong prognostic marker for long-term disease-free survival and might be helpful in defining follow-up strategies for patients with MTC.

Introduction

Medullary thyroid carcinoma (MTC) is a malignant tumor originating in the C-cells of the thyroid gland. MTC may occur sporadically (75%) or as part of an inherited cancer syndrome called multiple endocrine neoplasia type 2 (1,2). Hereditary MTC is caused by activating germline mutations of the *RET* (REarranged during Transfection) proto-oncogene, and genetic screening allows early diagnosis and treatment (3). Although MTC prevalence represents 2% of all thyroid malignancies, MTC accounts for 13.4% of all deaths attributable to thyroid cancer, highlighting the importance of identifying prognostic factors for persistent disease (4,5,6).

Tumor stage at diagnosis and the possibility of complete surgical resection are key factors leading to cure in MTC. Additional factors associated with MTC prognosis include age at diagnosis, extrathyroidal invasion, cervical lymph node metastasis, distant metastasis, and delayed or incomplete surgical treatment (4-12). Patients with MTC diagnosed by molecular screening, without regional metastases, or with stage I, II, and III disease may have an overall survival similar to that of the general population, whereas the presence of distant metastases predicts worse disease-specific survival (13).

The tumor-node-metastasis (TNM) classification system is used for postoperative MTC risk stratification and has shown a good correlation with disease-specific mortality (14). However, TNM staging incorporates both clinical and pathological information but does not include response to initial therapy, thus grouping together patients with similar pathological features but with different clinical responses at the same stage (15-17). Calcitonin, a highly sensitive and specific MTC biomarker, is widely used in the diagnosis, prognosis, and follow-up of patients with MTC and its measurement is recommended by current guidelines to assess patient follow-up (4,18,19). Also, calcitonin doubling time is often used to predict MTC prognosis, requiring at least 4 measurements with a 6-month interval between them to estimate doubling time (18).

Few studies have been conducted in recent years to evaluate the prognostic role of postoperative calcitonin (POCal). Jung *et al*, in 2016, published a 30-year retrospective analysis showing postoperative biochemical remission of serum calcitonin as the best predictive factor for recurrence-free survival in MTC (20). However, the study was conducted at 2 centers in the same city, had a short follow-up period (4.6 years), and considered the same calcitonin cutoff regardless of the measurement method used, thus reducing external validity. Saltiki *et al*, in a study published in 2014, suggested that POCal was the only predictor significantly associated with the 10-year progression of disease: POCal levels ≥4.65 pg/mL predicted disease persistence (sensitivity, 93.8%; specificity, 90%) and POCal levels ≥ 14.5 pg/mL predicted disease progression (sensitivity, 100%; specificity, 82%) (21). However, the study included only small MTCs (≤ 1.5 cm in size) and was conducted at a single center with a small number of patients (n = 128).

In the present study, we investigated the role of undetectable POCal as a marker for long-term disease-free survival in a large cohort of patients with MTC. As a secondary objective, we evaluated the performance of POCal to predict disease status after long-term follow-up.

Methods

Patients and study design

We evaluated a multicenter cohort of patients with MTC attending from 1997 to 2019 the Thyroid Unit at Hospital de Clínicas de Porto Alegre (HCPA) and the Endocrine Oncology Unit at the Brazilian National Cancer Institute (INCA), 2 tertiary-care teaching hospitals and referral centers for MTC treatment in Brazil. Since 1997, HCPA has been a referral center for the molecular testing for germline *RET* mutations in Brazil; therefore, patients referred to HCPA from other Brazilian centers for molecular investigation were also invited to participate. All patients with MTC had an anatomopathological diagnosis. Patients were excluded if they were lost to follow-up after initial therapy, had non-calcitonin-secreting tumors, or had only C-cell hyperplasia.

We obtained clinical and oncological data from 453 patients by medical record review. A questionnaire including clinical and oncological features and follow-up data is required from all patients followed up in other institutions. Of the 453 patients, 208 were followed up at HCPA, 111 at INCA, and 134 in other institutions or private practices.

The study was approved by HCPA Research Ethics Committee (CAAE 73671717.8.0000.5327), and written informed consent was obtained from each study participant and/or legal representative. All participants received pre- and post-test genetic counseling.

Institutional treatment protocol and follow-up

The MTC treatment protocol consisted of total thyroidectomy with central and/or bilateral neck exploration depending on serum calcitonin levels or evidence of suspicious lymph nodes on

preoperative neck ultrasound, according to current guidelines (4,18). Decisions about the extent of cervical lymph node dissection were made at the discretion of the surgical team. At the first evaluation, the following data were recorded for each patient: demographics, tumor characteristics (e.g., histological features, extension, and lymph node involvement), and treatment (e.g., surgery, radiotherapy, tyrosine kinase inhibitors, and other interventions).

The follow-up protocol consisted of an initial assessment at 3 to 6 months after surgery that included physical examination of the neck and measurements of serum calcitonin, carcinoembryonic antigen (CEA), and thyrotropin (TSH). Neck ultrasound was also performed during the first year of follow-up. Patients classified as disease-free (see *Outcomes* subsection below) were scheduled for annual visits that included physical examination of the neck and measurements of serum calcitonin, CEA, and TSH. Patients with persistent disease were scheduled for medical visits at least twice a year and evaluated for additional therapy as needed. Patients with calcitonin levels above 150 pg/mL were scheduled for additional imaging studies to evaluate distant metastasis. Follow-up duration was defined as the time from the thyroidectomy to the last medical visit to the clinic.

Postoperative serum calcitonin

Serum POCal was measured at 3 to 6 months after thyroidectomy. We divided patients according to POCal into 2 groups: undetectable (below the reference value of the method) or detectable calcitonin. We also performed a survival stratification analysis according to POCal levels (< 10 pg/mL; 10–100 pg/mL; > 100 pg/mL).

Molecular analysis

Genomic DNA was prepared from peripheral blood leukocytes by standard procedures. The DNA fragment of interest was amplified by polymerase chain reaction using a specific method to identify the presence of different *RET* mutations (2,3).

Laboratory analysis

The calcitonin measurement method has changed over the years. Radioimmunoassay was used from 1997 to 2005, immunochemiluminescence (Immulite 1000 – Siemens) from 2005 to 2010, chemiluminescence (Liaison - Diasorin) from 2010 to 2015, electrochemiluminescence (e602 – Roche) from 2015 to 2018, immunochemiluminescence (Immulite 2000 xpi – Siemens) from 2018 until present. Upon the implementation of each new technique, the necessary procedures for standardization and validation were performed. TSH levels were measured by electrochemiluminescent immunoassay (ADVIA Centaur XP; Siemens, Tarrytown, NY, USA). For statistical analysis, we considered the reference values for each method in a stratification model that included all measurement methods.

Outcomes

Disease status was defined based on clinical examination, serum calcitonin and CEA levels, neck ultrasound, and additional imaging tests when indicated. Patients were classified as disease-

free (undetectable calcitonin) or persistent disease. Those with biochemical disease (detectable calcitonin with no evidence of structural disease), structural disease (evidence of disease on imaging), and disease-related death were included in the persistent disease group.

Statistical analysis

Clinical and laboratory data were expressed as mean (SD) or median and 25th and 75th percentiles (P25–P75) for continuous variables, and as absolute numbers and percentages for categorical variables. We performed comparative analyses using unpaired Student's t-test, Mann-Whitney U-test, Fisher's exact test, or chi-square test as appropriate. We estimated survival using the Kaplan-Meier method and compared survival curves across groups by the log-rank test.

We investigated the factors associated with disease status during follow-up by grouping the patients into disease-free survival and persistent disease (biochemical, structural, or disease-related death). In univariate and multivariate regression analyses, we evaluated clinical variables, such as age, sex, tumor type, preoperative calcitonin, POCal, multifocality, tumor size, lymph node metastasis, and distant metastasis, as potential prognostic factors for MTC. To better evaluate POCal, we normalized calcitonin values by dividing the POCal value by the method's reference value (calcitonin value/lower limit of the reference value). We performed receiver operating characteristic (ROC) curve analysis to determine the optimal POCal cutoff, with a 95% confidence interval (CI). This cutoff was determined by the highest Youden index on the ROC curve. We analyzed the data in SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and set the level of significance at $P < 0.05$ for two-tailed tests.

Results

Patient characteristics

We included 453 patients with MTC with a mean age at diagnosis of 41.7 (SD, 18.4) years. Of these, we excluded 32 (16 due to lack of a histological diagnosis, 12 who were lost to follow-up, 2 with non-calcitonin-secreting tumors, and 2 who underwent prophylactic surgery for C-cell hyperplasia), for a total of 421 patients analyzed in the study (**Figure 1**).

The clinical and oncological features of the 421 patients with MTC are shown in **Table 1.** Most participants were women (60.6%), 176 (41.8%) had hereditary MTC, and 77 (18.3%) had MTC diagnosed by molecular screening. Median tumor size was 2.0 (range, 1.1–3.5) cm; 191 patients (45.4%) had lymph node metastasis and 78 (18.5%) had distant metastasis. Multifocal tumors accounted for 23.8% of cases, and 44 (10.5%) were associated with C-cell hyperplasia.

Hereditary MTC was diagnosed in 176 patients from 55 different kindred. We detected 18 different germline mutations in 12 codons and 7 exons. The most common mutation was C634Y, with a prevalence of 51.7% and affecting 91 patients from 10 kindred, followed by the M918T mutation detected in 21 patients from 16 kindred, of whom 6 belonged to the same family and 15 had *de novo* mutations. Thirty-five patients (8.3%) lacked information on *RET* genetic screening.

Postoperative serum calcitonin

Data on POCal measured at the first medical visit after surgery were available for 347 patients. At the first postoperative evaluation, 149 patients (42.9%) had undetectable POCal,

whereas 198 (57.1%) had detectable POCal (median POCal level of 438.0 [P25–P75, 87.1–1608.8] pg/mL).

Compared with patients with detectable POCal, more patients with undetectable POCal were women (71.8% vs 54.0%, P = 0.01), had MTC diagnosed by molecular screening (32.9% vs 10.1%, P < 0.001), had C-cell hyperplasia (28.3% vs 10.6%, P = 0.001), and had smaller tumors (1.5 vs 3.0 cm, P = 0.02). Also, patients with undetectable POCal had less lymph node metastasis (21.0% vs 76.7%, P < 0.001) and no distant metastasis (0% vs 35.2%, P < 0.001). The clinical and oncological features of patients with detectable vs undetectable POCal are shown in **Table 2**.

Postoperative serum calcitonin and disease outcome

Data on final disease status were available for 334 patients, 141 in the undetectable POCal group and 193 in the detectable POCal group. After a median follow-up of 8.5 (P25–P75, 2.0–13.8) years, 127 patients with undetectable POCal (90.1%) were disease-free, whereas 14 (9.9%) had persistent biochemical disease. Remarkably, no patient in the undetectable POCal group progressed to structural disease or disease-related death. All 14 patients with undetectable POCal and persistent disease had biochemical disease and low and stable calcitonin levels after 11.6 (P25–P75, 9.89–16.3) years of follow-up.

In the detectable POCal group, after a median follow-up of 6.9 (P25–P75, 2.1–12.5) years, 19 patients (9.8%) progressed to disease-free status, 50 (25.9%) had biochemical disease, 61 (31.6%) had structural disease, and 63 (32.6%) died of disease-related events.

At final follow-up, the analysis of a ROC curve with normalized POCal values identified a cutoff point of 1.0 as the optimal POCal cutoff to predict disease-free survival, with a sensitivity of 89.4%, specificity of 87.3%, and area under the curve of 0.928 (95% CI, 0.896–0.961) (**Figure 2**). The POCal cutoff was based on the highest Youden index (0.78).

Table 3 shows the results of univariate and multivariate analysis including age, sex, tumor type, preoperative calcitonin, POCal, multifocality, tumor size, lymph node metastasis, and distant metastasis. In multivariate analysis, POCal was a significant independent predictor of disease-free survival in MTC (hazard ratio, 4.66 [95% CI, 2.5–8.6]; P < 0.001).

Survival analysis

Our cohort had a disease-related death rate of 21.4% (79 patients). Most patients died in the first 10 years of disease (65.8%) (**Figure 3A**). All deaths occurred in the detectable POCal group.

The survival stratification analysis according to POCal levels (< 10 pg/mL; 10–100 pg/mL; > 100 pg/mL) showed better outcomes in the POCal < 10 pg/mL group, with a 25-year survival close to 100%. Interestingly, deaths in the 10–100 pg/mL group occurred in the first 10-15 years of disease; after this period, survival stabilized. **Figure 3B** shows MTC-specific survival stratified by POCal levels.

The intermediate group (POCal levels of 10–100 pg/mL) had a heterogeneous pattern, where some patients had a very good outcome and others had a poor outcome. In view of this, we compared patients with disease-free status to those with persistent disease within this group in an attempt to identify factors impacting prognosis. Patients who were disease-free had less lymph node metastasis (22.2% vs 69.6%, P = 0.001) and no MTC-related death (0% vs 21.9%, P = 0.01). The 2 subgroups did not differ in age at diagnosis, sex, tumor type, screening diagnosis, tumor size, C-cell hyperplasia, distant metastasis, multifocality, or follow-up duration (**Table 4**).

Discussion

Despite the advances in the management of MTC in recent years, uncertainty still exists about the best approach to follow these patients, particularly those with low or undetectable calcitonin levels after thyroidectomy. Our findings showed that all patients with undetectable POCal remained without evidence of structural disease throughout the follow-up period, and most of them were disease-free (90.1%) at long-term follow-up.

Traditional MTC prognostic factors are age at diagnosis, extrathyroidal invasion, cervical lymph node metastasis, distant metastasis, delayed or incomplete surgical treatment, and calcitonin and CEA doubling times (4-12). Calcitonin doubling time is an independent predictor of MTC prognosis, but at least 4 measurements over 2 years are required to calculate it.

Recently, several studies have proposed the use of a dynamic risk stratification system based on the best response to initial therapy to modify initial risk estimates by the classical TNM staging (15, 16, 17). Tuttle *et al*, after validating dynamic risk stratification for differentiated thyroid cancer, conducted a review of the literature to determine whether the previously proposed nomenclature for defining response to therapy in differentiated thyroid cancer could be applied to the management of MTC. They concluded that the intensity of follow-up and interventions can be tailored to constantly updated risk estimates in order to provide state-of-the-art management recommendations that are individualized for each patient (17). Lindsey *et al* analyzed 284 patients and found a recurrence rate of 4% among patients who achieved an excellent response to initial therapy, and a death rate of 54% among those with structural incomplete response to initial therapy. However, the median follow-up was just 5 years, a short time considering the indolent nature of the disease (15). Kwon *et al* conducted a similar study to evaluate response to initial therapy and

disease status at final follow-up in 120 patients with MTC. Patients with an excellent response showed the highest likelihood of being disease-free (88%), only 4% of patients with a biochemical incomplete response reached no evidence of disease, and no patients in the structural incomplete response group had disease-free status at final follow-up (16). In the present study, we showed that a single POCal measurement might determine a patient's long-term disease status.

Although serum calcitonin levels < 5 to 10 pg/mL are regarded as undetectable in the current literature and previous studies have considered 5 pg/mL as a cutoff for excellent response to initial therapy, we considered POCal undetectable if the levels were below the lower limit of the reference value for each method (ranging from < 3 to < 42 pg/mL) in order to increase reproducibility and external validity. This may explain why we obtained better results than other studies, as 90.1% of our patients with undetectable POCal had disease-free status and the remaining 9.9% had biochemical disease at final follow-up. Previous studies have reported 75%–80% of agreement between undetectable POCal and disease-free status at final follow-up.

Regarding mortality, our cohort had death rates similar to those of previous studies. Mathiesen *et al*, in a large cohort study, reported 5-, 10-, 15-, and 20-year disease-specific survival rates of 82% (CI, 76%–86%), 75% (CI, 67%–80%), 71% (CI, 63%–78%), and 69% (CI, 59%–77%), respectively (13). Another representative study of patients with MTC showed 5- and 10-year survival rates of 86% and 68%, respectively (22).

Of note, our cohort had a higher rate of hereditary MTC (41.8%) than that reported in the literature. This is probably because we are a referral center for *RET* genetic screening in Brazil.

Our study has some limitations. Most thyroidectomies were performed at a referral center by experienced surgeons, which probably led to better surgical outcomes. Another potential limitation is the use of different calcitonin measurement methods during follow-up; however, all

calcitonin methods were validated against the previous method and normalization strategies were used to reduce this bias. Therefore, regardless of the method used, if we multiply the lower limit of the POCal reference value by 1.0 (Youden index), we will have a sensitivity of 89.4% and specificity of 87.3% to predict disease-free survival at final follow-up. As expected in a retrospective study, some patients were lost to follow-up. However, the majority of patients were followed up at referral centers by multidisciplinary teams with expertise in MTC, which ensures the use of a similar therapeutic approach and follow-up strategy, thereby enhancing the validity of our data.

In conclusion, undetectable POCal is an independent predictor of disease-free survival in patients with MTC. Since POCal could predict the rate of disease remission and the risk of persistent biochemical/structural disease, it might also reduce the "over follow-up effect" with unnecessary surveillance, diagnostic tests, and medical appointments. From a practical standpoint, it may contribute to identifying, at the first postoperative evaluation, patients who are disease-free and those who require more careful follow-up.

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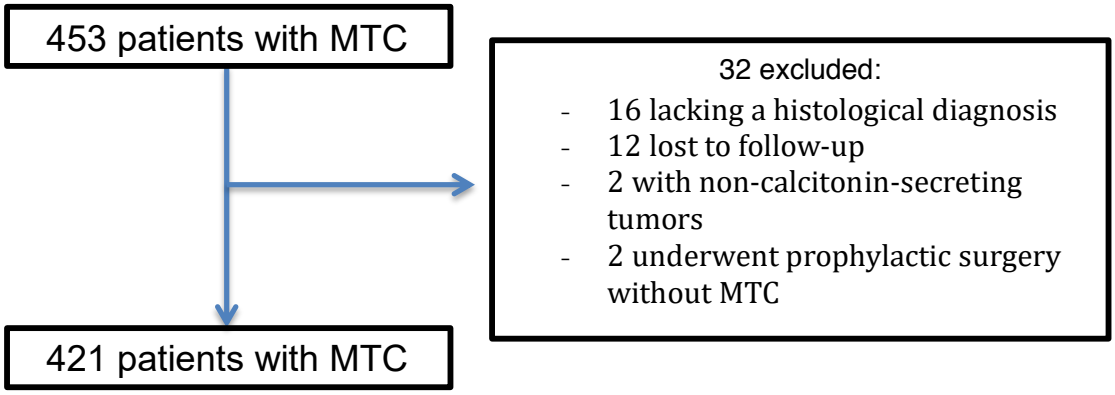

Figure 1. Flow chart of patients with MTC undergoing *RET* mutation analysis at HCPA.

MTC, medullary thyroid carcinoma; HCPA, Hospital de Clínicas de Porto Alegre.

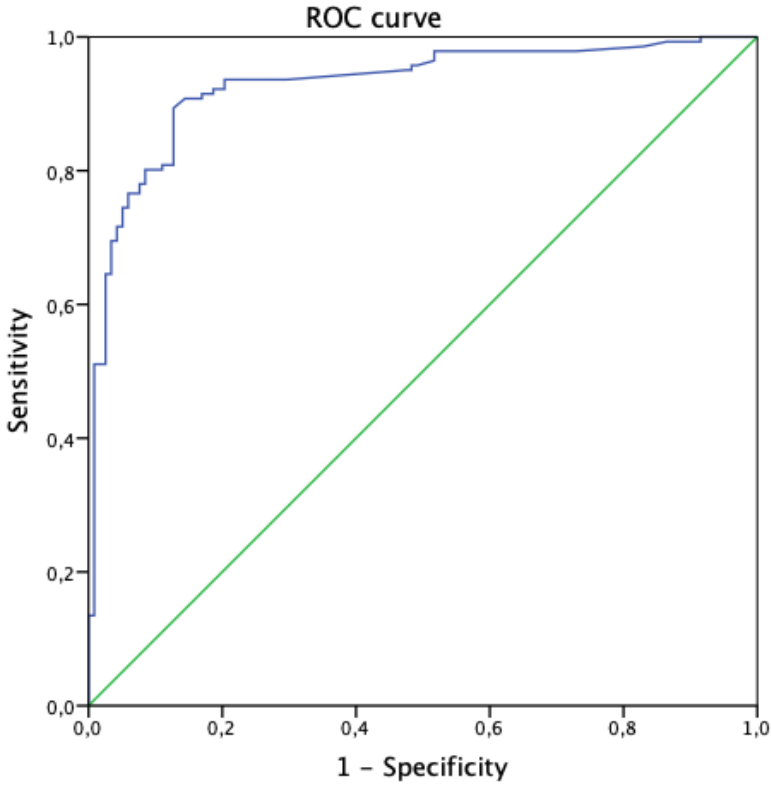

Figure 2.

Receiver operating characteristic curve of POCal levels normalized for reference values. There was a correlation with disease-free survival at final follow-up (Youden index = 1.0). Area under the curve = 0.93 (confidence interval, 0.90–0.96).

POCal, postoperative calcitonin.

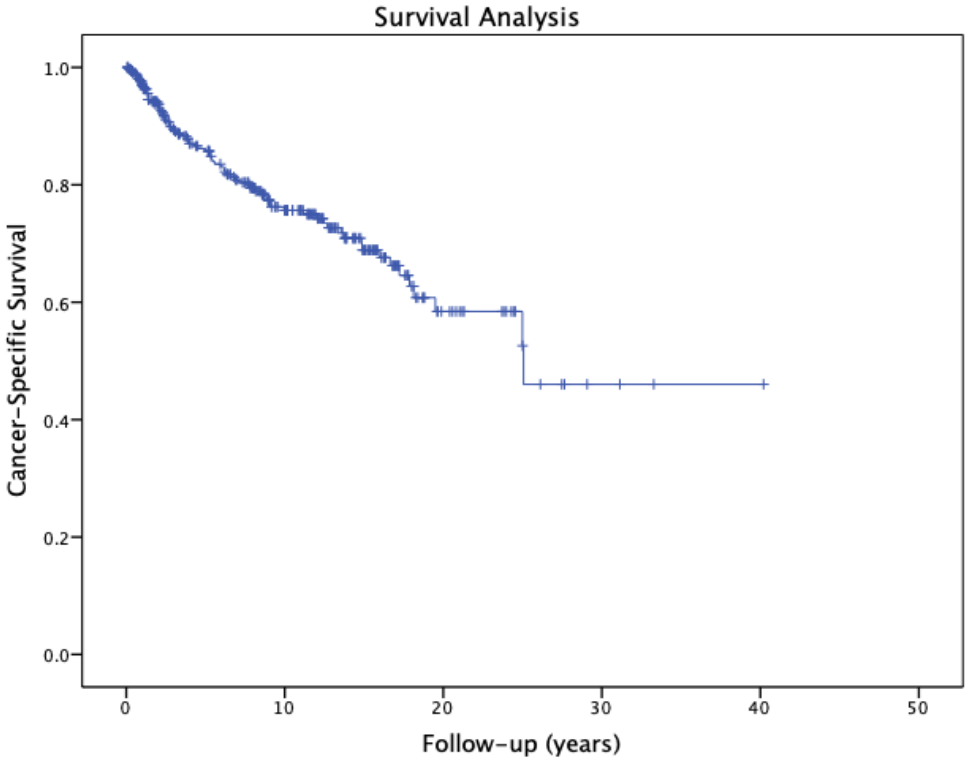

Figure 3A. Patient survival during follow-up.

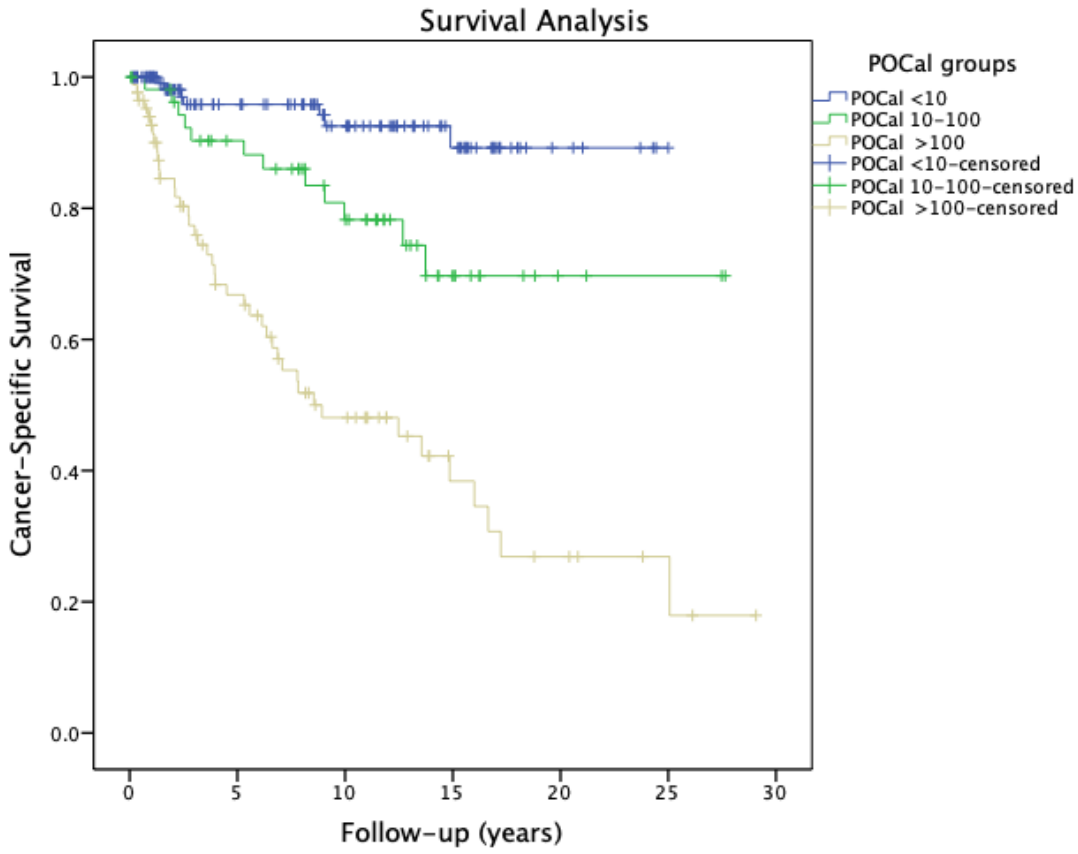

Figure 3B. Cancer-specific survival during follow-up (stratified by POCal levels in pg/mL).

POCal, postoperative calcitonin.

Table 1. Clinical and oncological features of 421 patients with MTC.

Characteristic	
Age at diagnosis (years)	41.7 ± 18.4
Female – n (%)	255 (60.6)
Tumor type Sporadic – n (%) Hereditary – n (%) Unknown – n (%)	 210 (49.9) 176 (41.8) 35 (8.3)
Screening diagnosis – n (%)	77 (18.3)
Preoperative calcitonin (pg/mL)	438.0 (87.1–1608.8)
Multifocal – n (%)	100 (23.8)
C-cell hyperplasia – n (%)	44 (10.5)
Tumor size (cm)	2.0 (1.1–3.5)
Lymph node metastasis – n (%)	191 (45.4)
Distant metastasis – n (%)	78 (18.5)
Follow-up (years)	7.8 (2.1–13.4)

Data are presented as mean ± SD or median (range) unless otherwise indicated.

MTC, medullary thyroid carcinoma.

Table 2. Clinical and oncological features of patients with MTC divided into undetectable and detectable POCal groups (n=347).

Characteristic	Undetectable POCal (n=149)	Detectable POCal (n=198)	P
Age at diagnosis (years)	39.6 (21.0–55.8)	43.6 (30.4–56.1)	0.48
Female – n (%)	107 (71.8)	107 (54.0)	0.01
Tumor type			0.17
Sporadic – n (%)	65 (43.6)	112 (56.6)	
Hereditary – n (%)	82 (55.1)	60 (30.3)	
Unknown – n (%)	2 (1.3)	26 (13.1)	
Screening diagnosis – n (%)	49 (32.9)	20 (10.1)	<0.001
Preoperative calcitonin (pg/mL)	133.0 (33.3–545.0)	1066.0 (435.0–3950.0)	0.50
Multifocal – n (%)	54 (44.6)	43 (42.2)	0.71
C-cell hyperplasia – n (%)	34 (28.3)	10 (10.6)	0.001
Tumor size (cm)	1.5 (0.7–2.5)	3.0 (1.8–4.1)	0.02
Lymph node metastasis – n (%)	30 (21.0)	138 (76.7)	<0.001
Distant metastasis – n (%)	0	64 (35.2)	<0.001
Follow-up (years)	8.5 (2.0–13.8)	6.9 (2.1–12.5)	0.40
Final disease status			
Disease-free – n (%)	127 (90.1)	19 (9.8)	<0.001
Persistent disease – n (%)	14 (9.9) *	174 (90.2) ♦	<0.001

Data are presented as median (range) unless otherwise indicated.

- * patients with stable persistent biochemical disease

- ♦50 biochemical disease / 61 structural disease / 63 disease-related deaths

MTC, medullary thyroid carcinoma; **POCal, postoperative calcitonin.**

Table 3. Univariate and multivariate analysis of prognostic factors for disease-free survival in MTC.

Characteristic	Disease-free	Persistent disease	Uni (P)	Multi (HR/CI; P)
Age at diagnosis (years)	38.6 (20.1–55.8)	42.0 (30.0–55.2)	<0.001	1.03 (1.0–1.0; p<0.001)
Female – n (%)	102 (66.7)	118 (55.9)	0.002	--
Sporadic – n (%)	69 (45.1)	108 (51.2)	<0.001	--
Preoperative calcitonin (pg/mL)	100.0 (29.0–549.5)	1175.0 (376.6–3669.0)	<0.001	--
Multifocal – n (%)	49 (44.1)	47 (43.9)	<0.001	--
Tumor size (cm)	1.5 (0.75–2.7)	3.0 (1.8–4.3)	<0.001	--
Lymph node metastasis – n (%)	25 (17.1)	151 (79.5)	<0.001	1.92 (1.2–3.0; p=0.005)
Distant metastasis – n (%)	0	73 (37.8)	<0.001	1.78 (1.3–2.5; p=0.001)
POCal – n (%)	127 (90.1)	14 (9.9)	<0.001	4.66 (2.5–8.6; p<0.001)

Data are presented as median (range) unless otherwise indicated.

MTC, medullary thyroid carcinoma; POCal, postoperative calcitonin; HR, hazard ratio; CI, confidence interval.

Table 4. Clinical and oncological features of patients with MTC and POCal levels of 10–100 pg/mL.

Characteristic	Disease-free (n=18)	Persistent disease (n=46)	p
Age at diagnosis (years)	39.6 ± 16.6	46.5 ± 15.9	0.44
Female (%)	12 (66.6)	25 (54.3)	0.54
Tumor type			0.18
Sporadic – n (%)	10 (55.6)	29 (63.0)	
Hereditary – n (%)	8 (44.4)	12 (26.1)	
Screening diagnosis – n (%)	5 (27.8)	7 (15.2)	0.42
Tumor size (cm)	2.5 (1.1–3.0)	2.3 (1.5–3.5)	0.45
Lymph node metastasis – n (%)	4 (22.2)	32 (69.6)	0.01
Distant metastasis – n (%)	0	8 (17.4)	0.14
C-cell hyperplasia – n (%)	4 (22.2)	2 (4.3)	0.11
Multifocal – n (%)	6 (33.3)	13 (28.3)	0.85
MTC-related death – n (%)	0	14 (21.9)	0.01
Follow-up (years)	13.0 (11.2–1)	7.9 (2.6–12.0)	0.55

Data are presented as mean ± SD or median (range) unless otherwise indicated.

MTC, medullary thyroid carcinoma; POCal, postoperative calcitonin

CONSIDERAÇÕES FINAIS

O CMT é um tumor raro e por isso ainda faltam estudos na literatura a respeito do seguimento desses pacientes. Por se tratar de uma doença mais agressiva que o CDT, muitas vezes os pacientes são submetidos a tratamentos mais invasivos e seguimentos com excesso de exames e consultas médicas, o que acaba reduzindo a qualidade de vida desses pacientes e onerando o sistema de saúde. Sendo assim, é fundamental tentar identificar aqueles pacientes que necessitam de tratamento precoce e diferenciado daqueles que podem seguir um acompanhamento com mais tranquilidade e sem necessidade de procedimentos/exames invasivos e de alto custo.

Nesta tese tivemos a oportunidade de aumentar o nosso conhecimento sobre o papel da calcitonina pós-operatória. Após análise de uma grande coorte de CMT, mostramos que a calcitonina pós-operatória indetectável é um preditor independente de sobrevida livre de doença e podemos já na primeira avaliação pós-operatória diferenciar os pacientes que estão livres de doença daqueles que necessitam de um acompanhamento mais intensivo.