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Biochemical Markers in the Follow-up of Medullary Thyroid Cancer

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Medullary thyroid cancer (MTC) shares biochemical features with other neuroendocrine tumors but the particular characteristics are largely unexplored. We investigated the biochemical neuroendocrine profile of MTC and whether specific markers could be useful in follow-up. In addition to the standard tumor marker calcitonin, plasma carcino-embryonic antigen (CEA), plasma catecholamines, (platelet) serotonin, chromogranin A, trypsinase, and urinary markers of catecholamine, histamine, and serotonin metabolism were prospectively determined in 46 patients with histologically proven MTC. Patients were divided according to the stage of disease: group 1, no evidence; group 2, stable disease (SD); and group 3, progressive disease (PD). Plasma dopamine was increased in the majority of the patients with SD and PD; however it did not correlate with extent of disease. Elevated plasma platelet levels of serotonin were only present in patients with multiple endocrine neoplasia 2 with SD or PD but did not differ between those groups. Histamine metabolites were elevated in 20% of patients with SD and PD. In addition to plasma calcitonin, only CEA and chromogranin A could differentiate between stable and progressive MTC. MTCs are capable of synthesizing catecholamines, serotonin, and histamine metabolites underscoring that MTCs have metabolic characteristics in common with other neuroendocrine tumors. Thus far, clinical usefulness and relevance seems limited. The most useful markers in the follow-up of MTC are plasma calcitonin and CEA.

Introduction

MEDULLARY THYROID CANCER (MTC) is a neuroendocrine tumor that, in addition to reliable tumor markers including calcitonin and carcino-embryonic antigen (CEA), secretes a large amount of other compounds such as calcitonin gene-related peptide, neuron-specific enolase, adrenocorticotrophic hormone, somatostatin, substance P, and vasoactive intestinal peptide (1,2), but these compounds have little clinical importance. The specific biochemical features of MTC, however, are still largely unexplored.

In the follow-up of MTC it is often difficult to firmly establish and subsequently localize suspected recurrences and/or metastases. Plasma calcitonin levels in patients with metastatic MTC may vary up to 30% if measured in short intervals, which makes it difficult to interpret progression (3). Characterizing the specific biochemical profile of MTC could provide an additional reliable tumor marker.

Neuroendocrine cells have a common ability of absorbing and decarboxylating monoamine precursors, thus producing biogenic amines such as catecholamines, serotonin, and his-

tamine (Fig. 1) and co-secreting tryptase and chromogranins (4,5). Because of these characteristics, Pearse (6) postulated the amine precursor uptake and decarboxylation (APUD) concept, according to which neuroendocrine tumors originate from a single neuroendocrine precursor cell. Cells of the APUD system include pancreatic islet cells, thyroid C-cells, gastrointestinal argentaffin cells, and adrenal chromaffin cells (7). On the basis of their confinement to the APUD concept, additional production of catecholamines and histamine by MTC is conceivable. MTCs have actually been reported to express various substances that reflect common neuroendocrine tumor functions including L-dihydroxyphenylalanine (L-DOPA) decarboxylase, an enzyme essential for catecholamine production (Fig. 1; 8,9). Previously, the presence of a common metabolic pathway for catecholamine synthesis has been described in carcinoid tumors and pheochromocytomas (10). Catecholamines have been detected in intracellular vesicles within MTC tumor cells but not in normal thyroid C-cells or follicular cells (11). Furthermore, the norepinephrine transporter is responsible for the cellular uptake of 6-[¹⁸F]-fluoro-L-3,4-dihydroxyphenylalanine

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FIG. 1. The major steps in catecholamine, serotonin, and histamine metabolism in neuroendocrine cells. The rate-limiting enzymes are underlined. Abbreviations: HVA, homovanillic acid; DOPAC, dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindole acetic acid; MIMAA, methylimidazole acetic acid; VMA, vanillylmandelic acid; MOPEG, 3-methoxy-4-hydroxyphenylethyleneglycol; VA, vanillic acid.

(F-DOPA), and up to 80% of MTCs concentrate this compound (12).

In light of the above, we investigated the biochemical profile of MTC and specifically addressed whether specific neuroendocrine tumor products could be used postoperatively as markers of disease progression.

Patients and Methods

Patients

In a prospective study over the period from January 2003 to August 2005, we evaluated 52 patients with histologically

proven MTC in different stages of the disease during regular outpatient visits generally 1–2 times a year. All patients were screened for mutations in the *RET* (REarranged during Transfection) gene. Twenty-six patients had sporadic MTC, 25 patients had multiple endocrine neoplasia (MEN) type 2A, and 1 patient MEN 2B. The latter were all confirmed by mutation analysis. Twenty patients with MEN 2 previously underwent adrenalectomy for pheochromocytoma and had no evidence of recurrence, as indicated by serum and urinary catecholamines and metabolites levels within reference limits. Six patients with MEN 2A had their adrenals *in situ* and were excluded from this study to avoid possible interference of

TABLE 1. PATIENT CHARACTERISTICS

	Sporadic MTC	MEN 2A/B	Total
No. of patients	26	20	46
Median age (range)	55 (25–79)	47 (25–75)	51 (25–79)
Male/female	11/15	11/9	22/24
No evidence of MTC	8	3	11
Stable MTC	7	10	17
Progressive MTC	11	7	18

MTC, medullary thyroid cancer; MEN 2A/B, multiple endocrine neoplasia type 2A/B

adrenal hormone production due to occult pheochromocytoma. Ultimately, we included 46 patients in this study. None of the patients had renal or hepatic dysfunction. Patient characteristics are depicted in Table 1. Previous thyroid surgery had included total thyroidectomy in all cases and central compartment lymph node dissection in 42 cases. An additional lateral dissection was performed in 28 patients (unilateral in 22 and bilateral in 6).

Patients were divided into three groups according to the stage of disease. Group 1 (11 patients) had no evidence of disease (NED) as indicated by undetectable basal and stimulated plasma calcitonin levels. Group 2 (17 patients) had stable disease (SD) as indicated by elevated levels of plasma calcitonin but no increase during at least three consecutive measurements over a period of 1–2 years and no clinical suspicion of recurrence. Group 3 (18 patients) had progressive disease (PD) as indicated by increasing plasma calcitonin levels or progressive MTC (occurrence of new lesions or obvious increase of tumor volume) on consecutive imaging methods.

In case of progressive MTC indicated by rising calcitonin levels (increase of 30% to 50%), an extensive imaging protocol was initiated. $^{99m}\text{Tc(V)}$ dimercaptosuccinic acid scintigraphy, ^{18}F -fluoro-2-deoxy-D-glucose positron emission tomography, magnetic resonance imaging, and ultrasonography of the neck and mediastinum were utilized to detect the source of calcitonin.

There was no difference between the groups regarding patients that used possible interfering medication such as β -blockers (two patients in group 1 [18%], three in group 2 [18%], and three patients in group 3 [17%]).

Samples

All samples were collected without dietary restrictions and without specific conditions (position, use of intravenous catheter) during routine outpatient visits. Venous blood was collected in 10 or 5 mL Vacutainer serum separator tubes (Becton Dickinson, Plymouth, UK). For determination of serotonin and catecholamines, tubes contained 0.12 mL of 0.34 mol/L EDTA solution. Samples for determination of calcitonin and catecholamines were put on ice without delay. The 24-hour urine samples were collected as described previously (10). We divided compounds into five groups: tumor markers currently used in the follow-up (calcitonin and CEA), substances that are co-secreted with biogenic amines (chromogranin A and tryptase), catecholamines, indoles, and markers of histamine metabolism. All samples were run in duplicate.

Analytical methods

Creatinine Urine creatinine concentrations, used to express analyte concentrations in terms of urinary excretion rate, were measured by a picric acid method as described previously (13).

Calcitonin and CEA Plasma calcitonin levels (reference values 0.3–12 ng/L) were measured by enzyme-linked immunosorbent assay (Sangui Biotech Inc., Santa Ana, CA). Plasma CEA levels (reference values 0.5–5 $\mu\text{g/L}$) were measured by chemiluminescent microparticle immunoassay (Abbott Laboratories, Abbott Park, IL).

Chromogranin A and tryptase Plasma chromogranin A levels (reference values 20–100 $\mu\text{g/L}$) were determined by solid phase two-site immunoradiometric assay (CIS Bio International, Gif-sur-Yvette, France). Plasma tryptase levels (reference value <11.4 $\mu\text{g/L}$) were determined by a fluoro-enzyme immunoassay (Pharmacia, Woerden, the Netherlands).

Catecholamines As markers of catecholamine metabolism we used plasma and urine epinephrine, norepinephrine, and dopamine and urine metanephrine, normetanephrine, 3-methoxy-thyramine (3-MT), homovanillic acid (HVA), vanillylmandelic acid (VMA), vanillic acid (VA), 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG), and dihydroxyphenylacetic acid (DOPAC). Plasma and urine epinephrine (reference values: plasma, 0.12–0.22 nmol/L; urine, <10 $\mu\text{mol/mol}$ creatinine), norepinephrine (reference values: plasma, 2.65–4.31 nmol/L; urine, <30 $\mu\text{mol/mol}$ creatinine), and dopamine (normally not detectable in plasma; reference value urine, <300 $\mu\text{mol/mol}$ creatinine) were determined using high-pressure liquid chromatography with electrochemical detection as described previously (14). The urinary excretion of fractionated total normetanephrine (reference values 64–260 $\mu\text{mol/mol}$ creatinine), metanephrine (reference values 33–99 $\mu\text{mol/mol}$ creatinine), and 3-MT (reference values 45–197 $\mu\text{mol/mol}$ creatinine) was determined using extractive derivatization and stable isotope gas chromatography with mass fragmentographic detection (15). The urinary excretion of HVA (reference values 1.1–5.5 mmol/mol creatinine), VMA (reference values 0.5–2.5 mmol/mol creatinine), VA (reference values 5–15 mmol/mol creatinine), MOPEG (reference values 0.4–1.5 mmol/mol creatinine), and DOPAC (reference values 0.4–1.2 mmol/mol creatinine) was determined by gas chromatography with flame ionization detection as described previously (16).

Indoles As markers of serotonin metabolism we used plasma tryptophan (reference values 40–70 $\mu\text{mol/L}$) and plasma platelet serotonin content (reference values 2.8–5.4 nmol/ 10^9 platelets). Furthermore, we used urine 5-hydroxyindole acetic acid (5-HIAA, reference values 0.8–3.8 mmol/mol creatinine). An extended description of the sampling procedures and analytical methods was described previously (10,17).

Histamine metabolites Urine methyl-histamine (reference value <150 $\mu\text{mol/mol}$ creatinine) and methylimidazole acetic acid (MIMAA, reference values 0.9–1.9 mmol/mol

creatinine) were determined by stable isotope gas chromatography with mass fragmentographic detection as described previously (13,15).

Statistics

For each patient all available data of a single analyte were averaged. Group differences between the biochemical values were evaluated using the Kruskal–Wallis test. Whenever differences were found, the individual group differences were tested with the Mann–Whitney U test, using Bonferroni correction. Correlation between biochemical markers was calculated using Pearson’s correlation coefficient. Sensitivity of an analyte was calculated as number of patients with a true positive result/(number of patients with a true positive result + number patients with a false negative result). Patients who were cured (group 1) were used as true negatives.

Reference values, as defined by previous studies that included healthy control subjects (described in Kema *et al.* [13]) or by the manufacturer of the different assays, were used to estimate the sensitivity of the markers. Differences with a two-sided *p* value less than 0.05 were considered significant.

Results

Biochemical profile of MTC

During the study period, a total of 134 plasma samples were obtained from all 46 patients. Urine samples (*n* = 87) were obtained from 34 patients (74%). Of these 34 patients, 18 had sporadic MTC and 16 had MEN 2. None of the patients underwent treatment that resulted in significant lowering of the plasma calcitonin and CEA levels. Data for plasma and urine compounds were calculated from a median of two measurements (range 1–9) per patient.

The biochemical profile regarding co-secreted compounds, catecholamines (divided into dopamine and norepinephrine metabolites), indoles, and histamine metabolites is shown in Fig. 2. Since calcitonin was used to establish diagnosis and stage of disease, it was not included in the figure.

Co-secreted compounds Plasma chromogranin A levels were elevated in 12 patients (one patient in group 1, three in group 2, and eight in group 3), most with advanced MTC. Plasma tryptase levels remained within reference levels in all patients.

FIG. 2. Concentrations of co-secreted compounds, dopamine metabolites, norepinephrine/epinephrine metabolites, indoles, and histamine metabolites for 46 patients with medullary thyroid cancer (MTC). Since calcitonin was used to establish diagnosis and stage of disease, it is not included in the figure. Concentrations are expressed as multiples of upper reference limit. Compounds are measured in urine unless stated otherwise. The vertical line represents the upper reference limit. The boxplots depict the 95% range of observations, lower quartile, median, upper quartile, and outliers (open dots). Note that dopamine is usually not detectable in plasma and therefore only elevated levels are depicted. Abbreviations: MIMAA, methylimidazole acetic acid; 5-HIAA, 5-hydroxyindole acetic acid; MOPEG, 3-methoxy-4-hydroxyphenylethyleneglycol; VA, vanillic acid; VMA, vanillylmandelic acid; 3-MT, 3-methoxytyramine; HVA, homovanillic acid; DOPAC, dihydroxyphenylacetic acid; CgA, chromogranin A.

Catecholamines As shown in Fig. 2 plasma dopamine levels were elevated in 22 patients, 18 of them with SD or PD (group 2 or 3). In general, dopamine is not detectable in plasma, but in this study it was detected in four patients with NED (group 1). In one of these four patients plasma norepinephrine was also elevated indicating increased sympathetic activity. In the other three patients all levels of plasma and urine catecholamines were within reference limits.

DOPAC levels were elevated in nine patients and HVA in two, all with SD or PD.

Five patients (all with sporadic MTC) had (slightly) elevated plasma norepinephrine levels and 15 patients had elevated plasma epinephrine. Consequently, urine norepinephrine levels were elevated in 13 patients, normetanephrine, and metanephrine levels in four patients, VMA levels in three patients and MOPEG in four patients.

Indoles Seven patients had elevated platelet serotonin levels (up to 17.4 nmol/10⁹ platelets). All had MEN 2 (six MEN 2A, one MEN 2B). None of these patients had low tryptophan levels. Five of the seven MEN 2 patients had progressive MTC (in four, metastatic MTC was biopsy proven).

Histamine metabolites Six patients had elevated MIMAA levels up to 3.9 mmol/mol creatinine. However, none of the patients had symptoms of serotonin or histamine excess (flushing, diarrhea, abdominal cramping, wheezing, angioedema).

Biochemical markers by type and extent of MTC

Type MTC There were no significant differences between sex ($p=0.578$), age ($p=0.557$), and extent of disease ($p=0.228$) between patients with sporadic MTC or MEN 2.

We found a remarkable correlation between elevated platelet serotonin levels and MEN 2 ($p=0.0004$). Patients with sporadic MTC had significantly higher plasma epinephrine ($p<0.0001$) as well as urine epinephrine ($p=0.001$) and metanephrine ($p<0.0001$). There were no significant differences between the median plasma concentrations of the tumor markers calcitonin and CEA, the co-secreted compounds chromogranin A and tryptase, plasma and urine catecholamines, and plasma and urine indoles and histamine metabolites by type MTC.

Extent of MTC In Table 2 the estimated sensitivities of the tumor markers calcitonin and CEA and the other co-secreted compounds are shown. Since calcitonin is an established tumor marker for MTC, sensitivity was put on 100%.

Levels of catecholamines, indoles, and histamine metabolites could not discriminate between different stages of disease activity (NED, SD, or PD). Only CEA ($p<0.0001$) and chromogranin A ($p=0.051$) reached (or approached) significance. In Fig. 3 concentrations of CEA and chromogranin A in patients from group 1, 2, and 3 are shown. Since calcitonin was used to establish stage of disease, it was not included in the figure. Chromogranin A was mainly elevated in patients with an extended tumor burden. All markers were also correlated with calcitonin. Only CEA and chromogranin A correlated significantly (Table 2).

FIG. 3. Concentrations of plasma carcino-embryonic antigen (CEA) and chromogranin A in the three groups with no evidence of medullary thyroid cancer (MTC, group 1), stable disease (group 2), and progressive disease (group 3). Since calcitonin was used to establish diagnosis and stage of disease, it is not included in the figure. The boxplots depict the 95% range of observations, lower quartile, median, upper quartile and outliers (open dots). Concentrations of CEA and chromogranin A are expressed in µg/L. y-axis is on a logarithmic scale. Upper dotted line: upper limit of normal; lower dotted line: lower limit of normal. p values are represented in the plots.

Discussion

This study advances knowledge in the specific neuroendocrine biochemical behavior of MTC. We have demonstrated that MTCs can synthesize catecholamine, serotonin, and histamine metabolites. However, plasma calcitonin, CEA, and to a lesser extent chromogranin A are the only clinically useful tumor markers to differentiate between stable and progressive MTC.

A substantial number of patients with MTC have increased concentrations of circulating free catecholamines. In 22 (56%) of our patients, plasma dopamine levels were increased, but urinary dopamine levels were normal. This marked discrepancy between elevated plasma and urinary dopamine levels could be explained by the fact that urine dopamine concentrations are the consequence of both clearance of circulating dopamine and (mainly) renal production and excretion of dopamine. Therefore, dopamine produced by the tumor comprises only a small part of the dopamine excretion in urine.

Eighteen patients with disease activity showed detectable plasma dopamine levels suggesting that tumor cells could be responsible for the dopamine production. Detectable dopamine levels in the four patients with NED might be explained by increased sympathetic activity, although levels of other catecholamines were within reference limits in these patients.

Patients with sporadic MTC had significantly higher plasma epinephrine as well as urine epinephrine and metanephrine compared to patients with MEN, although they did not always exceed the reference limits. These differences are

TABLE 2. ESTIMATED SENSITIVITY OF ANALYTES AND CORRELATION WITH CALCITONIN

	<i>Patients with abnormal levels (patients measured)</i>	<i>Estimated sensitivity (%)</i>	<i>r with calcitonin</i>	<i>p</i>
Tumor markers				
Calcitonin (ng/L)	35 (35)	100	1	<0.0001
CEA ($\mu\text{g/L}$)	21 (35)	60	0.93	<0.0001
Co-secreted compounds				
Chromogranin A ($\mu\text{g/L}$)	12 (35)	31	0.51	<0.0001
Tryptase ($\mu\text{g/L}$) ^a	0 (34)	—	-0.13	0.394
Catecholamines				
Dopamine and metabolites				
Plasma dopamine (nmol/L)	22 (35)	56	0.17	0.250
Urinary dopamine ($\mu\text{mol/mol creatinine}$)	0 (32)	—	0.12	0.499
DOPAC (mmol/mol creatinine)	9 (32)	26	0.01	0.947
HVA (mmol/mol creatinine)	2 (32)	4	0.22	0.221
3-MT (mmol/mol creatinine) ^a	0 (32)	—	0.16	0.369
Norepinephrine/epinephrine and metabolites				
Plasma norepinephrine (nmol/L)	5 (46)	11	0.11	0.474
Plasma epinephrine (nmol/L)	15 (46)	34	0.08	0.595
Urinary norepinephrine ($\mu\text{mol/mol creatinine}$)	13 (34)	41	0.05	0.774
Urinary epinephrine ($\mu\text{mol/mol creatinine}$) ^a	0 (34)	—	0.08	0.652
Normetanephrine ($\mu\text{mol/mol creatinine}$)	4 (34)	10	0.20	0.255
Metanephrine ($\mu\text{mol/mol creatinine}$) ^b	Total 21 (34)	14	0.29	0.102
	• Low 17			
	• Elevated 4			
VMA (mmol/mol creatinine)	3 (31)	11	0.16	0.407
VA (mmol/mol creatinine) ^b	Total 21 (32)	4	0.05	0.794
	• Low 20			
	• Elevated 1			
MOPEG (mmol/mol creatinine)	4 (32)	11	0.12	0.526
Indoles				
Tryptophan ($\mu\text{mol/L}$) ^b	13 (46)	14	0.00	0.991
	• Low 6			
	• Elevated 7			
Serotonin (nmol/10 ⁹ platelets) ^b	25 (46)	20	0.00	1
	• Low 18			
	• Elevated 7			
5-HIAA (mmol/mol creatinine) ^a	0 (31)	—	-0.07	0.702
Histamine metabolites				
Methyl-histamine ($\mu\text{mol/mol creatinine}$)	2 (32)	7	0.17	0.345
MIMAA (mmol/mol creatinine)	6 (31)	23	0.25	0.173

^aNo abnormal values, therefore, sensitivity could not be calculated.

^bSensitivity calculated for elevated levels only.

Abbreviations: CEA, carcino-embryonic antigen; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; VMA, vanillylmandelic acid; VA, vanillic acid; MOPEG, 3-methoxy-4-hydroxyphenylethyleneglycol; 5-HIAA, 5-hydroxyindole acetic acid; MIMAA, methylimidazole acetic acid

likely to be caused by the absence of adrenals in patients with MEN 2.

Except for plasma and urine epinephrine and metanephrines, there was no difference between patients with sporadic MTC and MEN 2-associated MTC in metabolites of the catecholamine pathway.

Catecholamine production by tumor cells of MTC is likely because the elevated urine catecholamine metabolites in our patients indicated sustained increased levels of circulating catecholamines. Also, elevated plasma dopamine, DOPAC and plasma and urine norepinephrine metabolite levels were found in the patients with disease activity (group 2 and 3) irrespective of the tumor type. Moreover, dietary and drug-dependent influences (18) are unlikely given the sample size of the study population and the low frequency of interfering medication. The increased levels of plasma dopamine, DO-

PAC, and norepinephrine metabolites detected in the present study indicate that both *de novo* synthesis of dopamine and norepinephrine from tyrosine and partial *de novo* synthesis from accumulated dopamine from extracellular sources are possible.

Our findings support an earlier report about catecholamines detected in intracellular vesicles in MTC tumor cells and not in normal thyroid C-cells or follicular cells (11) MTCs have been reported to express various substances that reflect common neuroendocrine tumor functions including tyrosine hydroxylase (19) and L-aromatic amino acid decarboxylase (8,9). It may be that the dopamine producing capacities of MTC are due to malignant transformation of the C-cells. Unfortunately, we could not correlate advanced stage of disease to an increase of catecholamines and their metabolites.

In addition to catecholamine-excreting capacities of MTC, we found increased plasma platelet serotonin levels in approximately 15% of MTCs and only in patients with MEN 2. Conflicting findings mentioned either increased (20–22) or undetectable (9) amounts of serotonin in serum and tissue of several patients with MTC and comparable serum serotonin or urine 5-HIAA levels in patients with MTC and healthy controls (23,24). The high number of patients with elevated serotonin levels found in this study could be explained by the use of platelet serotonin which is more sensitive to measure serotonin synthesis than urine 5-HIAA or serum serotonin (13,25).

In MTC platelet serotonin sometimes increases dramatically up to levels (over three times the upper reference limit) that were thought to be pathognomonic for carcinoid. A concurrent carcinoid might be responsible for the elevated platelet serotonin levels. However, this is very unlikely since we had no clinical evidence for the presence of a carcinoid after an active search for disease in all patients with progressive MTC. The lack of symptoms of serotonin excess could be explained by the low levels of 5-HIAA, which are more correlated to symptoms than platelet serotonin levels.

MTCs are capable of serotonin biosynthesis via tryptophan hydroxylase (26) and aromatic amino acid decarboxylase (8). Electron microscopic immunocytochemistry revealed that in MTC serotonin is stored in secretory vesicles together with calcitonin (27). The number of these vesicles is highly variable which may explain the differences in serotonin secretion.

The almost exclusive occurrence of serotonin elevation in MEN 2 patients might indicate that sporadic and hereditary MTC have different biochemical profiles. The elevated serotonin secretion could then be a sign of some form of dedifferentiation of MTC that is specific for the hereditary form.

In approximately 20% of patients MTCs possess features of histamine metabolism excreting levels of MIMAA that mimic concentrations that are usually found in mastocytosis. Histamine synthesis is a one-step process that requires histidine decarboxylase, which is expressed in MTCs (28). However, no symptoms of mast cell disease were observed. Histamine production occurs in other APUDomas as well, but in MTCs it is probably not an important feature (29,30).

Clinicians should be aware of the neuroendocrine features of MTC described in this study. Increased concentrations of circulating free catecholamines in MTC may lead to confusion in the laboratory diagnosis of catecholamine-secreting tumors such as pheochromocytoma. Furthermore, secretion of serotonin and histamine may account for some of the symptoms of the carcinoid syndrome (29,30). It is possible that release of these compounds from MTCs can cause side effects, such as arrhythmia and perioperative carcinoid crisis in a subset of patients (22,31,32).

The clinical relevance of our findings may be expanded with respect to future molecular imaging methods of MTC that depend on expression of catecholamine uptake and storage systems. F-DOPA, for instance, is taken up by neuroendocrine cells, via the cell membrane norepinephrine transporter and translocated into storage vesicles via the vesicular monoamine transporter, before undergoing conversion to 6-[¹⁸F] fluoronorepinephrine catalyzed by dopamine β-hydroxylase (33). F-DOPA positron emission tomography (F-DOPA-PET) identified MTC metastases in about 80% of patients (12). It might even be that determining plasma

dopamine could predict whether performing F-DOPA-PET would be successful. However, this needs to be further elucidated.

With respect to the clinical usefulness of the different compounds as tumor markers, the present study is essentially a negative study. We have shown that, in addition to plasma calcitonin levels, only CEA and, to a lesser extent, chromogranin A can differentiate between stable and progressive MTC, a conclusion that was reached by others as well (35–37). Based on our data, neither CEA nor chromogranin A is superior to calcitonin. However, plasma calcitonin levels can vary, especially in patients with a high tumor burden (3) and calcitonin, CEA, and chromogranin A can behave quite differently especially in dedifferentiated MTC (38). Although plasma calcitonin levels are highly sensitive for the detection of early recurrences, in advanced progressive MTC CEA may be a useful tumor marker (39) and should therefore always be measured in the follow-up of MTC. However, routine CEA measurements are not recommended by some authors (40). Chromogranin A can offer additional information about the course of the disease in some selected patients with MTCs that do not express calcitonin and CEA and may be helpful, especially in patients in an advanced stage of MTC, despite the low sensitivity (36,37,41).

In conclusion, MTCs express a number of products from the catecholamine, serotonin, and histamine synthesis pathway suggesting that MTCs and other neuroendocrine tumors indeed have metabolic characteristics in common as postulated in the APUD concept, but a clinical application of these compounds cannot be established.

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