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AGGRESSIVE CLINICAL COURSE OF MEDULLARY THYROID MICROCARCINOMA

Abstract: Medullary thyroid carcinoma is a form of neuroendocrine tumor that arises from parafollicular C cells which produce calcitonin. In addition to calcitonin, these cells produce smaller amounts of other peptides, including carcinoembryonic antigen (CEA), which is used as a nonspecific tumor marker in the follow-up of patients with this tumor. MTC is a rare thyroid tumor and occurs three times more often in women than in men. It can occur in two forms, sporadic (80%) and familial form (20%). The familial form can occur alone or in association with other endocrine tumors within MEN 2A and MEN 2B syndromes. The sporadic form most often occurs in the fifth and sixth decades of life. The familial form is inherited autosomally dominantly, most often based on a mutation in the RET protooncogene located on chromosome 10. C-cell hyperplasia is considered to be a premalignant lesion, which precedes medullary carcinoma. Medullary carcinoma metastasizes very early. We presented a patient with a sporadic form of MTC which appeared at a typical age. Initial values of both baseline and stimulated calcitonin were not in the range for suspected MCT, but due to persistent increases in calcitonin, with elevated baseline (63 pg / mL) and higher stimulated calcitonin (96 pg / mL), the patient was referred for surgical treatment. Due to the strong correlation of calcitonin values with tumor size, the initial calcitonin values were expected to be low because the tumor was 3 mm in size. The histopathological diagnosis was C-cell hyperplasia. However, due to the fact that nodular C-cell hyperplasia is histopathologically difficult to distinguish from medullary microcarcinoma, based on the persistent increase in calcitonin levels, the patient was likely to already have metastatic disease at the time of thyroidectomy. Definitive diagnosis was made by liver biopsy. Therapy with tyrosine kinase inhibitors was introduced, and calcitonin levels strarted to decrea, but there is an increase in carcinoembryonic antigen, which is a poor prognostic parameter.

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Key words: Medullary thyroid carcinoma, C-cell hyperplasia, aggressive disease course, metastases, calcium stimulation test, tyrosine kinase inhibitors, calcitonin, carcinoembryonic antigen

Case report

The 56-year-old patient was admitted to the Clinic for Endocrinology, Diabetes and Metabolic Diseases of the University Medical Center for metastatic medullary thyroid carcinoma.

She first contacted an endocrinologist in November 2018. due to micronodus in the thyroid gland, when calcitonin 4.4 ng / L was measured. In July 2019. control calcitonin was marginally higher, 8.1 ng / L, and calcium test was performed. The test did not show an excessive increase in calcitonin level (Table 1). In the further course, an increase in base calcitonin is registered (Table 1). In March 2020, Ultrasound examination of the thyroid gland describes two to three microcysts in the distal part of the right lobe (RL) as well as hypoechoic micronodus 3mm. In the middle part of the RL, a posteriorly irregular iso- to heteroechoic nodule with an irregular, thickened, hypoechoic halo without CD signal, 5x5x6mm in diameter. In the left lobe (LL) several microcysts. In the middle part of the jugular chain on the right, an enlarged, hypoechoic lymph node (LN) with a diameter of 5x9 mm with central vascularization, it cannot be said with certainty whether it is reactive or pathologically altered LN. TIRADS DL 4 LL October 2, 2020. a needle biopsy of theRL nodule was performed, CP finding: in the analyzed puncture there is a small amount of colloids, numerous erythrocytes, moderately numerous lymphocytes, scarce striated cell stems, few single and grouped follicular cells. Bethesda II. Serum calcium and phosphate levels, as well as catecholamine metabolites in 24 h urine were normal (Table 1).

Due to an increase in calcitonin levels, surgical treatment was indicated, and on December 30, 2020. total thyroidectomy was performed. Intraoperatively, the nodule in the RL was found and was described as parenchimal, with a diameter of about 3 mm. PH finding: RL: C- cell hyperplasia and papillary microcarcinoma. Postoperatively calcitonin levels continiues to increase (Table 1). She was admitedd to the hospital again in May 2021, for disease staging. At that time MRI of the abdomen and CT of the lungs showed multiple metastatic lesions in the liver and lungs, MRI of the spine showed no secondary deposits. During this hospitalization, the liver lesion biopsy was performed: PH dg: carcinoma medullare glandulae thyreoideae metastaticum in hepate. Immunohistochemical (IHH) analysis: Calcitonin (+), Synaptophysin (+), mCEA (+) and Thyreoglobulin (-). The patient was referred for assessment for stereotactic radiotherapy (STRT) of the liver and lung lesions, but it was concluded that STRT was not indicated due to the number of lesions. Somatostatin receptors

scintigraphy (99mTc-Tektrotyd) showed accumulation in the described lesions along the segmental bronchi for the lower lobe, spiculed lesions in the right anterobasal segment and some hypodense lesions in the liver that indicates discrete expression of somatostatin receptors (Krenning score 1). Contrast-enhanced CT of the endocranium showed no clear signs of expansive lesions.

At that point, procedure for tyrosine kinase inhibitor therapy has been initiated.

Reevaluation of the patient's condition was performed in January 2022. The results of the laboratory analysis are shown in Table 2. **ECG**: sin. rhythm, fr 88 / min, PQ interval 0.16s, no ST and T changes were seen. **Ultrasound examination of the neck**: there is no residual tissue in the thyroid bed. In the proximal part of the jugular chain on the right (level II), a suspicious LN, 4x11 mm in diameter, with suspected microcalcifications was seen. In the distal part of the jugular chain on the left, two pathologically altered Lnswere seen, the larger one was 9x13 mm in diameter. In the distal part of the jugular chain on the right, two pathologically altered LNs with a larger diameter of 3x5 mm were seen. **Chest CT scan:** in the anterobasal segment of the lower right lung lobe there is an infiltrative central necrotic lesion, 41x30x20 mm in diameter, with calcifications centrally and the broad contact with the interlobar and diaphragmatic pleura. In the right hilus, there are necrotic pathologically altered lymph nodes up to 12 mm in diameter, tracheobronchialy, on right up to 13mm, paratrachealy on right up to 12mm. The trachea and bronchial tree are of normal diameter and branching, with no noticeable wall thickening and intraluminal contents. The mediastinal structures showed normal presentation. No pleural effusion. The vertebral body Th6 has an oval zone of osteosclerosis with a diameter of 20 mm. The liver is affected in all segments by hypodense vaguely limited lesions with a hyperdense edge of the appearance of secondary deposits. The adrenal glands are of normal morphology, without nodular thickenings. **MRI of the abdomen:** on the basal sections through the chest, a soft tissue lesion in the mediobasal segment of the lower right lobe is observed. The liver is of regular size, with multiple previously verified lesions of restrictive diffusion, MR characteristics of secondary deposits, the largest is in IIS / IIIS that consists of several confluent lesions, with total diameter of 72x43mm, and in IS with diameter of 27x27 mm. A simple 7 mm diameter cyst in IIIS as well as several microcysts were seen. The gallbladder is an elongated, distended, with diameter of 75x45mm (KKx LL), with denser traction sediment at the bottom up to 22 mm in diameter. Multiple solitary LNs are observed, the largest being hepatogastric up to 10 mm in diameter. No ascites is seen. **MR of Th spine:** The finding corresponds to degenerative changes without secondary deposits. **Craniogram:** No pathological changes were seen on the bones of the skull roof. In February 2022, tyrosine kinase inhibitor therapy (vandetanib) was introduced, followed by a significant decrease in calcitonin, but an increase in CEA (Table 1).

Discusion

Medullary thyroid carcinoma (MCT) is a form of neuroendocrine tumor that arises from parafollicular C-cells derived from the neural crest, which produce calcitonin. In addition to calcitonin, these cells produce smaller amounts of other peptides, including carcinoembryonic antigen (CEA), which is used as a nonspecific tumor marker in the follow-up of patients with this tumor. MTC covers 3-12% of all thyroid cancers and occurs three times more often in women than in men (F: M = 3: 1) (1). It can occur in two forms, sporadic (80%) and familial form (20%). The familial form can occur alone or in association with other endocrine tumors within MEN 2A syndrome (in addition to medullary carcinoma, includes primary hyperparathyroidism and pheochromocytoma) and MEN 2B (includes pheochromocytoma, mucosal ganglioneuroma, and mafanoid habitus). The sporadic form most often occurs in the fifth and sixth decades of life. The hereditary form can occur at any age, most often at a younger age, while the isolated form usually occurs in the fifth decade of life. The familial form is inherited autosomal dominantly, most often based on a mutation in the RET protooncogene located on chromosome 10. The sporadic form of medullary carcinoma has a worse prognosis than the familial form in MEN2 syndrome, and the tumor within MEN 2B syndrome shows the greatest biological aggression.

C-cell hyperplasia is considered to be a premalignant lesion, which precedes medullary carcinoma. It is much more common in hereditary form, but it also occurs in some sporadic cases of MCT. There are two forms of C-cell hyperplasia that have clearly different pathogenetic mechanisms, physiological or reactive C-cell hyperplasia and neoplastic C-cell hyperplasia, which is carcinoma in situ (2,3). When neoplastic C-cell hyperplasia shows a predominantly nodular type of growth, it is difficult to distinguish it from medullary microcarcinoma, and this differentiation is extremely important given the metastatic potential of microcarcinoma (4). In the familial form of MCT, tumors are more often multicentric, bilateral, multitype and usually smaller at the time of diagnosis, while the sporadic form is usually unilateral.

Histologically, numerous variants are observed, but the histological type of medullary carcinoma is not important for the prognosis of the disease. In addition to histological characteristics, IHH analysis is necessary for diagnosis, and staining is performed on tumor-specific marker - calcitonin and panneuroendocrine markers – chromogranin (CgA), neurospecific enolase (NSE) and synaptophysin. Calcitonin is detected immunocytochemically in 95–100% of MCT, and CEA in 77-100%. CEA is sensitive, but not specific for medullary cancer. Tumors with poorer calcitonin positivity have more aggressive biological behavior (5,6). Medullary carcinoma in addition to calcitonin secretes other biological markers: CEA, CgA, CGRP (calcitonin gene related peptide), NSE, serotonin, ACTH, vimentin, cytokeratin, glucagon with CEA as a good prognostic marker. Calcitonin levels correlate with tumor size. Postoperatively, the level of calcitonin indicates residual tissue and serves as a parameter for

monitoring the possible progression of the disease. MTC has limphatic spread in the neck and mediastinum and hematogenous spread when it gives distant metastases in the lungs, bones and liver. Medullary carcinoma metastasizes very early. Lymph node metastases are present in up to 43% of MTC ≤ 10 mm, and 20% are incurable. (7)

Diagnostic procedures in case of suspicion of MCT, after visualization of the nodule, are primarily focused on the detection of tumor products, ie calcitonin as the main biological marker. Routine measurement of serum calcitonin may reveal C-cell hyperplasia or medullary carcinoma. However, normal values of calcitonin do not exclude a C-cell tumor. In the case of elevated baseline values of calcitonin, a calcium stimulation test is performed (8, 9). In case of non-conclusive or subjective findings of fine needle biopsy of a suspicious nodule, IHH examination of needle washings on calcitonin, CgA and CEA with evidence of absence of thyroglobulin is advised (10).mmmnn,

Routine measurement of serum calcitonin in patients with nodular / multinodular goiter is the best method for early identification of suspected medullary carcinoma. It has been shown that patients with routinely determined calcitonin had less advanced stage of MTC, and in the postoperative course more frequent normalization of serum calcitonin and better long-term prognosis compared with patients without routine determination of calcitonin. The reason for this outcome is the diagnosis of MTC at an early stage, which is essential in the treatment of this cancer (11). Indications for routine calcitonin measurement have not yet been universally proposed by scientific societies. In 2006, ETA recommended the measurement of calcitonin in the initial treatment of thyroid nodules (12), newer ATA and AACE / ACE / AME guidelines are vague regarding the routine measurement of calcitonin (neither recommended, nor against) (13, 14). However, patients diagnosed and treated at a later stage require lifelong control measurements of calcitonin, costly diagnostic treatment for residual disease or additional surgical procedures, and the cost of these additional tests and reoperations could be significantly greater than those for detecting a single case at an early stage. Insufficient sensitivity and specificity of calcitonin measurements still calls into question the value of its routine use due to the low prevalence of medullary thyroid cancer (0.32% in patients with nodular thyroid disease). The disadvantage of routine calcitonin measurement is the high variability of calcitonin levels depending on the laboratory / type of test (15,16), as well as the finding of slightly higher basal calcitonin levels than the normal range due to other causes. Therefore, in the interpretation of the results, it is necessary to exclude other possible causes of elevated calcitonin levels (use of proton pump inhibitors, chronic renal failure, pseudohypopituirism, ectopic production of calcitonin by non-thyroid neuroendocrine tumors, hypergastrinemia, chronic thyroiditis, interference with heterophilic antibodies…). In the study of Fugazzola et al. from 2020. the refined cut-offs for basal and calcium stimulated calcitonin that could potentially indicate MTC were presented. Those are: baselin calcitonin levels greater than 30 ng / L for women, and greater than 34 ng / L for men, while for calcium-stimulated calcitonin were > 79 ng / L for women

and> 466 ng / L for men. Baseline calcitonin has been shown to be highly accurate, although some cases have only been diagnosed by a stimulation test. By combining baseline calcitonin levels either below or above the cut-offs with stimulated calcitonin above the cut-off level, all MTC cases were correctly identified. The median and mean values were 21.38 and 15 ng / L (range 2.8–53.7) for tumors <5 mm, 52.26 and 58.8 ng / L (range 5.6–126) for tumors of 5–10 mm, 227.6 ng / L (range 12.9–1860) for tumors ≥ 10 mm (P <0.001). A significant correlation was found between tumor size and basal calcitonin levels. On the other hand, there was no significant correlation between tumor size and stimulated calcitonin levels (17)

The 2015 ATA guidelines for the treatment of MCT (10) advise total thyroidectomy, dissection of cervical LN depending on serological, visualization, and intraoperative findings. External beam radiotherapy (EBRT) is applied to the neck if there is an evidence of extensive local disease, residual disease, or extrathyroid spread. It is known that MCT, as a neuroendocrine tumor, expresses somatostatin receptors (SSTR) and peptide receptor radionuclide therapy can have both diagnostic and therapeutic value. Tyrosine kinase inhibitor (TKI) therapy has its place in patients with progressive symptomatic metastatic disease. TKIs are small molecule inhibitors that specifically target and inhibit the action of tyrosine kinases. Because RET protooncogene is a form of tyrosine kinase receptor, TKIs can inhibit RET protein phosphorylation leading to regulation of its downstream pathways and consequent inhibition of tumor growth. Today, several TKIs are used: imatinib, gefitinib, motesanib, sunitinib, sorafenib, axitinib, apatinib, pazopanib, lenvatinib, vandetanib, and cabozantinib. Local cryo-, thermo- or chemo-ablation of liver metastases can also be used successfully. (18, 19)

We presented a patient with a sporadic form of MTC which occurred at a typical age. Initial levels of both, baseline and stimulated calcitonin were not in the range for suspition of MCT, but due to persistent increases in calcitonin with elevated baseline (63 pg / mL) and higher stimulated calcitonin (96 pg / mL), the patient was referred for surgical treatment. Given the correlation of calcitonin levels with tumor size, the initial calcitonin levels were expected to be low because the tumor was 3 mm in size. The lesion in the thyroid gland was characterized as C-cell hyperplasia, which is a premalignant lesion, less common in the sporadic form of the disease, and the revision of the PH findings did not find a criterion for changing the diagnosis to medullary thyroid cancer. However, due to the fact that nodular C-cell hyperplasia is difficult to distinguish histopathologically from medullary microcarcinoma, based on a persistent increase in calcitonin levels, it is most likely that the patient already had metastatic disease at the time of thyroidectomy (then suspected LN in the right jugular chain). The definitive diagnosis was made by biopsy of the liver. Due to the wide distribution of the disease, the STRT target was not advised, so the patient continued treatment with a tyrosine kinase inhibitor, that resulted in a decrease in calcitonin levels, but with a simultaneous increase in CEA, which is an unfavorable indicator of the disease course.

Table 1.

	Calcitonin (ng/L)		
2018.	4,4 ng/L		
2019.g. July	8,1 ng/L		
Calcium stim. test 2019.	9,5…33,6…32,7…31,2…26,9…25,7 ng/L		
2019. December	13,2 ng/L		
2020. January	22,4 ng/L		
2020.February	25,2 ng/L		
Calcium stim. test 2020. November	63 ng/L…96 ng/L		
2020. December	84 ng/L; dilution 1:10 - 94.8 ng/L, RIA In needle washing 85.8 ng/L (RIA), 92,1 ng/L		metanephine 0.44umol/24h normetanephrine 2.0umol/24h
2020. December	**Thyroidectomy**		
2021. April	151 ng/L		
2021. May	303,4 ng/L	362 ng/L	**CEA**(µg/L)
2021. August	1167 ng/L		20,9 µg/L
2022. January	7789 ng/L		216,0 µg/L
2022. February	**Introduction of vandetanib therapy**		
2022. March	7559 ng/L		473,0 µg/L
2022. May	2000 ng/L		497,5 µg/L
2022. Jun	>2000 ng/L		915,3 µg/L

Table 2.

RBC	5.14	Glc	4.4	Albumin	39	gamaGT	176	PO4	1.0
HGB	140	Urea	5.1	Chol	4.67	LDH	516	Mg	0.79
HTC	0.409	Creatinine	53	HDL	0.92	CK	46	UIBC	30.5
MCV	80.0	eGFR	> 60	LDL	3.05	Na	133	TIBC	36.0
WBC	9.7	Bilirubin	6.8	Tg	1.54	K	2.8	PTH	< 3.0
PLT	424	Bilirubin dir	4.2	AST	37	Cl	90	vitamin D	29
CRP	47	Ac. uricum	660	ALT	24	Ca	3.15	TSH	4.6
HbA1c	5.9 %	Proteins	68	ALP	258	Ca++	1.48	fT4	16.8

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