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Clinical genetic of medullary thyroid carcinoma

Genetyka kliniczna raka rdzeniastego tarczycy

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S u m m a r y

Medullary thyroid carcinoma (MTC) is a neuroendocrine malignant neoplasm, developing from the parafollicular thyroid cells. These cells, arising from the neural crest, migrate from the fifth branchial cleft into thyroid gland during the embryogenesis. They secrete calcitonin, a peptide hormone, facilitating calcium transition from blood to bones.

MTC occurs in the sporadic and hereditary form, which presence is related to the *RET* protooncogene germline mutations. Hereditary form of MTC can be divided into familial medullary thyroid carcinoma (FMTC) without any other endocrinopathies and as a part of multiple endocrine neoplasia type 2 (MEN 2).

Multiple endocrine neoplasia type 2a (MEN 2a), named also as Sipple syndrome, is characterized by the presence of MTC, pheochromocytoma (in about 50% of patients) and parathyroid adenomas or hyperplasia (15-25% of patients). The diagnosis of MEN 2b syndrome is more unequivocal because of its characteristic clinical status and a typical *RET* mutation. In this syndrome, MTC develops the most quickly, even in young children. A characteristic symptom is the presence of mucosal neuromas and neurogangliomatosis of the distal intestinal tract. Pheochromocytoma develops later, in about half of patients. Parathyroid adenomas are absent.

In this chapter the actual state of knowledge concerning the molecular basis of MTC hereditary form, its relation to localization of *RET* mutations and clinical disease status are presented. Diagnostic and therapeutic procedures in hereditary MTC and the way of proceeding in a presence of germline *RET* mutation are discussed Short guidelines about management in hereditary MTC are also given.

S t r e s z c z e n i e

Rak rdzeniasty tarczycy (RRT) jest neuroendokrynnym nowotworem złośliwym, wywodzącym się z okołopęcherzykowych komórek C. Komórki te pochodzą z grzebienia nerwowego, w czasie rozwoju płodowego migrują z V kieszonki skrzelowej do tarczycy, gdzie produkują kalcytoninę. Kalcytonina jest hormonem peptydowym, ułatwiającym przejście wapnia z krwi do kości.

Rak rdzeniasty tarczycy występuje w postaci sporadycznej oraz dziedzicznej, której wystąpienie związane jest z obecnością mutacji germinalnych protoonkogenu RET. Dziedzicznemu RRT mogą nie towarzyszyć żadne inne objawy i mówi się wówczas o rodzinnym raku rdzeniastym tarczycy (ang. *familial medullary thyroid carcinoma*, FMTC). Częściej jednak dziedziczny RRT jest objawem zespołu wielogruczołowego typu 2 (ang. *multiple endocrine neoplasia type 2*, MEN 2).

Zespół wielogruczołowy typu 2A (MEN 2A), zwany również zespołem Sipple'a, charakteryzuje się skojarzeniem RRT z guzami chromochłonnymi nadnerczy (u około 50% chorych) i gruczolakami lub hiperplazją przytarczyc (u około 15-25% chorych). Rozpoznanie zespołu MEN 2B jest daleko bardziej jednoznaczne, tak ze względu na obraz kliniczny jak i charakterystyczne mutacje. W tym zespole RRT rozwija się najszybciej, jeszcze u małych dzieci i towarzyszą mu nerwiaki błon śluzowych oraz przerost zwojów przywspółczulnych śluzówki jelita grubego. Guzy chromochłonne nadnerczy występują później i ujawniają się u około połowy chorych, natomiast nadczynność przytarczyc nie występuje.

W artykule przedstawiono aktualny stan wiedzy na temat molekularnego podłoża dziedzicznej postaci RRT oraz zależność między lokalizacją mutacji punktowej RET i obrazem klinicznym choroby. Omówiono również postępowanie diagnostyczne i lecznicze w dziedzicznej postaci RRT oraz postępowanie w razie wykrycia nosicielstwa mutacji protoonkogenu RET. Jednocześnie w podsumowaniu podano krótkie wskazówki dotyczące postępowania w przypadku wykrycia dziedzicznej postaci RRT.

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INTRODUCTION

Medullary thyroid carcinoma (MTC) is a neuroendocrine malignant neoplasm, arising from the parafollicular thyroid cells. According to the world literature its discovery is associated with the name of Hazard (1). However, it is worthy to emphasize that the first information concerning this type of cancer was published earlier, in the Polish oncological journal "Nowotwory" by professor Laskowski who named it "carcinoma hyalinicum".

C-cells arise from the neural crest and migrate from the fifth brachial cleft into thyroid gland during the embryogenesis. They secrete calcitonin, a peptide hormone, facilitating calcium transition from blood to bones.

MTC is usually localized in the middle-upper part of lateral thyroid lobes, where accumulation of parafollicular cells is the greatest. Histologically, neoplastic C-cells are usually disposed in small groups or nests, separated by thin fibro-vascular layers or rarely they form trabecular, insular or solid structures. C-cell hyperplasia may be seen in surrounding thyroid tissue. A typical feature, however not always observed, is the presence of amyloid in the background. MTC cells show a positive reaction for calcitonin. Thus, regardless of classical histopathological examination, immunohistochemistry with the use of anticalcitonin antibodies is obligatory to state MTC diagnosis. MTC in more than 90% of cases causes significant increase of serum calcitonin level (Ct). Therefore, its assessment in patients suspicious for cancer significantly facilitates the diagnosis.

MTC spreads via lymphatic and blood vessels. Lymph node metastases, involving central neck compartment at the beginning and later lateral neck lymph nodes, present at diagnosis in 50-75% of cases, are often bilateral with extracapsular invasion. However, neck ultrasound (US) may fail to identify nodal metastases, mostly with reference to central neck compartment. The degree of lymph node involvement usually correlates to the primary tumor diameter. Distant metastases, via blood route, are usually localized in the liver, lung and bones.

In cases of locally advanced MTC a thyroid tumor may infiltrate adjacent tissues such as blood vessels, nerves, neck muscles, trachea and esophagus.

The first MTC symptom, observed in most patients is a nodule of thyroid gland, gradually increasing, showing different dynamics of growth, mostly indolent and generally painless. Few to several percent of subjects sometimes demonstrate chronic diarrhea, the first sign of advanced disease, which is related to excessive secretion of biologically active biogenic amines and peptides by the tumor. Dyspnea, obstacles feeling when swallowing or even dysphagia occur in patients with locally advanced disease. Cough, liver enlargement, bone pain (both spontaneous and palpable) and rapid weight loss may accompany disseminated MTC.

The percentage of MTC patents showing genetic predisposition is relatively high 20-25%, whereas in selected populations, intensively screened, even up to 30% (2, 3).

HEREDITARY MEDULLARY THYROID CANCER

Hereditary MTC may occur as an isolated disease – familiar medullary thyroid carcinoma (FMTC) or it constitutes a part of multiple endocrine neoplasia type 2 (MEN 2) syndrome (tab. 1).

MEN 2a is also known as Sipple syndrome in which MTC, affecting nearly 100% of patients, is accompanied by pheochromocytoma (~ 50% of all cases) and/or parathyroid hyperplasia (~ 15-25% of all cases). MTC usually constitutes the first symptom of Sipple syndrome and occurs within the first two decades of life, whereas pheochromocytoma is usually diagnosed later and rarely is the first sign of the disease. Primary hyperthyroidism appears as the last one, so its prevalence varies depending on the age of the investigated population.

Table 1. Hereditary medullary thyroid carcinoma: clinical manifestations.

Symptome	FMTC	MEN 2A	MEN 2B
Medullary thyroid cancer	> 95%	> 95%	> 95%
Pheochromocytoma	–	~ 50%	~ 50%
Primary hyperparathyroidism	–	15-60%	–
Typical appearance: elongated face with a big jaw, mucous neuro-matosis, colon hyperganglionosis causing symptoms similar to Hirschprung's disease	–	–	100%

In a non classical form of MEN 2a syndrome cutaneous lichen amyloidosis (CLA) or Hirschsprung's disease rarely may also occur (2).

Pheochromocytoma is characterized by an episodic high blood pressure (paroxysmal hypertension) with concomitant tachycardia sometimes accompanied by pallor and/or excessive perspiration. Unrecognized/untreated pheochromocytoma may lead to sudden death and in fact it is much more life threatening than MTC itself, especially taking into consideration its relatively low aggressive course in patients with MEN 2a syndrome.

Primary hyperparathyroidism results in hypercalcemia secondary to excessive PTH secretion. PTH increases bone resorption, therefore osteoporosis belongs to early symptoms of the disease in contrary to brown bone tumors observed much later. Among typical features of advanced primary hyperparathyroidism are renal stones, peptic ulcer disease, pancreatitis, gastrointestinal disturbances, cardiovascular and psychiatric disorders. Untreated hyperparathyroidism may lead to hypercalcemic crisis.

Because MTC represents the most frequent initial diagnosis, the differentiation between FMTC and a classical MEN 2a syndrome requires a long follow-up as pheochromocytoma may occur after years and never affects all members of a family. According to the literature the diagnosis of truly FMTC is unequivocal when at least four family members are diagnosed with MTC only. If the number of patients is lower than 4, unclassified hereditary MTC is diagnosed, as even DNA tests do not allow to distinguish unequivocally between FMTC and MEN 2a syndrome (see below).

The diagnosis of MEN 2b syndrome is much more unequivocal because of its characteristic clinical appearance as well as a typical *RET* mutation. In MEN 2b syndrome MTC develops much earlier than in FMTC or MEN 2a syndrome, even in small children. Pheochromocytoma occurs later and affects ~ 50% of patients, while primary hyperparathyroidism is not a part of this syndrome. Phenotype features of MEN 2b syndrome such as: elongated face with a big jaw and very prominent lips are so characteristic that an experienced clinician may state the diagnosis during the first patient's visit. Neuromatosis of tongue margins and oral mucosa constitute a very typical feature that is seen during physical examination. Some patients also present marfanoid habitus.

Hereditary nature of some MTC cases has been known since the 60-ties of XXth century. At this time, to early diagnose MTC in family members serum calcitonin concentration in pentagastrin stimulation test used to be measured (4). Such tests were carried out in all family members up to the age of 14. To avoid false positive results (pentagastrin may stimulate an increase in serum calcitonin level even in healthy subjects, mostly in young male patients) the value above 100 pg/ml were considered as diagnostic. This assessment of serum calcitonin concentration sometimes made possible to properly characterize a genetic predisposition in family members and therefore facilitated to investigate a linkage between the incidence of MTC and genetic markers of the disease.

THE *RET* PROTOONCOGENE AND MTC

A gene responsible for hereditary MTC was localized in a centromeric region of chromosome 10 in 1987. It was identified as the *RET* protooncogene and simultaneously its mutations, responsible for FMTC, MEN 2a and MEN 2b syndromes, were described in 1993 (5-7).

The *RET* protooncogene encodes a membranous receptor tyrosine kinase. Its extracellular part includes a ligand binding site, cadherin like domain and cysteine rich domain (fig. 1).

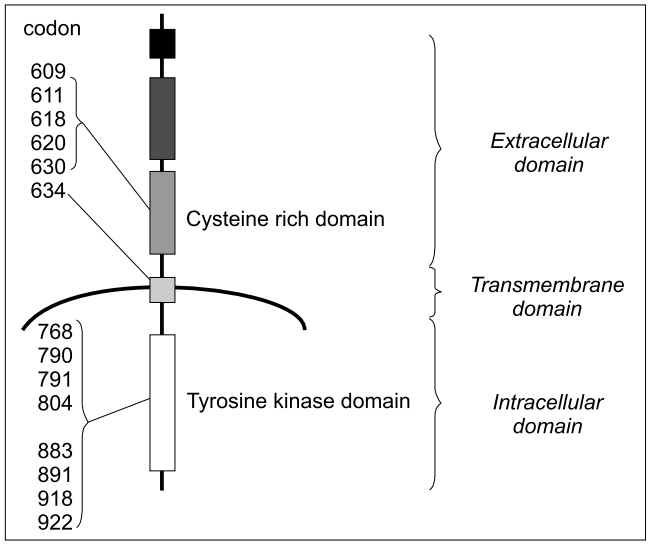

Fig. 1. The scheme of a structure of RET receptor tyrosine kinase with localization of codons undergoing activating mutations.

A short transmembrane domain, fixes the protein within the cell membrane. The third, an intracellular part contains closely located two tyrosine kinase domains. The structure of RET protein strictly corresponds with a structure of other growth factor receptors (such as EGF), which are in fact receptor tyrosine kinases. A ligand responsible for a growth signal transduction by the RET protein is a small neuropeptide, glial cell-derived neurotrophic factor (GDNF). This peptide does not directly bind to the RET protein but to other membrane protein known as α GDNF receptor (currently GFRα-1) acting as RET co-receptor (fig. 2). The consequence of receptor activation is its autophosphorylation leading to downstream induction of MAP kinases and transcription of genes involved in cell proliferation.

The *RET* protooncogene mutations, responsible for MTC development, are activating mutations resulting in overactive RET protein (2). The *RET* protooncogene consist of 21 exons. However, mutations occur only in a few of them and most are point mutations (fig. 1 shows their localization with reference to encoded protein). They mainly concern cysteine rich domain of the receptor extracellular part, close to the cell membrane. *RET* codon 634, localized in exon 11 is mostly (75-80% of all hereditary MTC cases) subject to mutations (tab. 2). More than 90% of them result from replacement of amino acid cysteine with arginine, tyrosine or tryptophan (8, 9).

The classical MEN 2a syndrome is the most likely when *RET* codon 634 mutations are present, whereas other mutations are related to significantly lower probability of pheochromocytoma development and most often cause FMTC without other endocrinopathies (tab. 2).

Mutations in codon 918 of the *RET* protooncogene (exon 16) concern the tyrosine kinase domain. Because other proteins are phosphorylated, phenotypes of MEN 2a and MEN 2b syndromes are different. RET overactivation is observed also in peripheral nerves (neurinomas of tongue and mucous of oral cavity, colon hyperganglionosis). MTC occurs earlier and is characterized by the more aggressive course. However, there is no parathyroid hyperplasia (12, 13).

Mutations in codons 768, 790, 791, 804 and 891 (exons 14, 15, 16, 17), also in intracellular domain of the RET protein, are rare and their transforming potential is low, except of mutations in *RET* codon 790 observed in FMTC and MEN 2a syndrome (14). They mainly lead to the development of FMTC, which may occur relatively late, in 4th or 5th decades of life. With respect to mutation in codon 791 it is believed that its penetrance might be incomplete. Whereas, the other *RET* gene mutations are characterized by complete penetrance. Thus, the diagnosis of germinal mutation of the *RET* gene is equal to more than 90% probability of MTC development. *RET* codon 791 mutation carriers show a variable risk of MTC development and at least in some of them clinically overt MTC occurs relatively early. The polymorphic nature of nucleotide change at position 791 is discussed in the literature.

Table 2. The localization of the *RET* protooncogene mutation responsible for hereditary MTC (10, 11).

Codon/ Exon	Syndrome	Incidence % (13)	Incidence (39)
609/10	MEN 2A/FMTC MEN 2A/ Hirschsprung's disease	0-1	0%
611/10	MEN 2A/FMTC	2-3	2.5%
618/10	FMTC/MEN 2A MEN 2A/ Hirschsprung's disease	3-5	12%
620/10	FMTC/MEN 2A MEN 2A/ Hirschsprung's disease	6-8	3%
630/11	MEN 2A/FMTC	0-1	0%
634/11	MEN 2A MEN 2A/CLA	75-85	42%
635/11	MEN 2A	rarely	not investigated
637/11	MEN 2A	rarely	not investigated
768/13	FMTC	0-1	1%
790/13	FMTC/MEN 2A	0-1	2.5%
791/13	FMTC	0-1	16%
804/13	MEN 2A/FMTC	0-1	8%
883/15	MEN 2B	rarely	rarely
891/15	FMTC	rarely	not investigated
918/16	MEN 2B	3-5	12%
922/16	MEN 2B	rarely	not investigated

GENOTYPE-PHENOTYPE RELATIONSHIP IN HEREDITARY MTC

The relationship between localization of *RET* point mutation and clinical manifestation of the disease is clear. From the genetic point of view MEN 2a syndrome and FMTC are close and currently they are rather treated together – FMTC is considered as a one form of MEN 2a syndrome, while MEN 2b syndrome is considered separately due to both its typical phenotype and initiating mutation. The likelihood of a typical MEN 2a syndrome strongly depends on a type of *RET* mutation – it is very high in a case of *RET* codon 634 mutation (the clinically overt syndrome with primary hyperparathyroidism occurs most often when arginine replaces cysteine), lower when *RET* mutation is localized in exon 10 and very low in case of exons 13 and 15 (18).

DNA TESTS

At a clinical level there are no differences between hereditary and sporadic MTC. Therefore, DNA analysis assessing the presence of a germinal *RET* mutation is obligatory in all MTC subjects. The global risk of a germinal *RET* mutation detection in Polish population of MTC patients is about 10% even if there is no positive family history and no features of MEN 2a syndrome are present (3). Figure 3 presents the algorithm of screening for the RET protooncogene mutation (19, 20). The order of codon analysis in the RET protooncogene is related to the frequency of a particular RET mutation. Thus, the routine analysis starts from codon 634. This analysis may be carried out by the use of PCR/RFLP technique. However, despite of a negative result of this examination the analysis of other codons should be performed as almost half of newly diagnosed MTC show mutation in exons 13-15. Due the same reason DNA analysis should not be omitted in elderly subjects – some *RET* mutations are characterized by the late MTC onset.

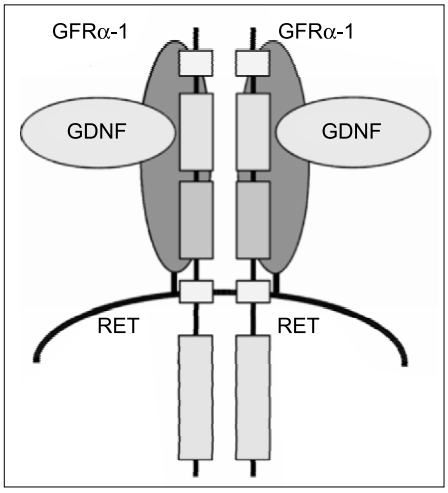

Fig. 2. Physiological activation of the RET tyrosine kinase.

The assessment of mutations in exons 10 and 13-15 requires DNA sequencing. An analysis to detect *RET* codon 918 mutation is usually carried out on the basis of patient's phenotype, however in some cases phenotypic features of MEN 2b syndrome are not so clear and only DNA test allows to state the proper diagnosis. It should be emphasized, that small *RET* gene size and a limited number of its characteristic mutations are the factors, which facilitate DNA analysis. In Poland this analysis is performed in several centers[1]. Patients and their families benefit from the identification of a germinal *RET* mutation. In a particular patient it allows for the assessment of the risk of pheochromocytoma and hyperparathyroidism. Thus it determines the frequency of screening analyses. Simultaneously, detection of a genetic predisposition to MTC definitely obligates to DNA testing on family members. The risk of mutation in first-degree relatives is 50%. Our studies show at least a one detected mutation carrier falls on a one case of newly diagnosed hereditary MTC (3, 21). The likelihood of early cancer diagnosis varies depending on a type of *RET* mutation and age of family members – in some of them clinically overt MTC is detected, whereas other subjects demonstrate elevated serum calcitonin level only (basal or after pentagastrin stimulation test) without any changes in thyroid ultrasound (US). This early screening allows for the detection of asymptomatic gene carriers and asymptomatic MTC.

[1]DNA tests are performed in each MTC patient in Institute of Oncology in Gliwice, Poland (tel. +48 (32) 278 93 01).

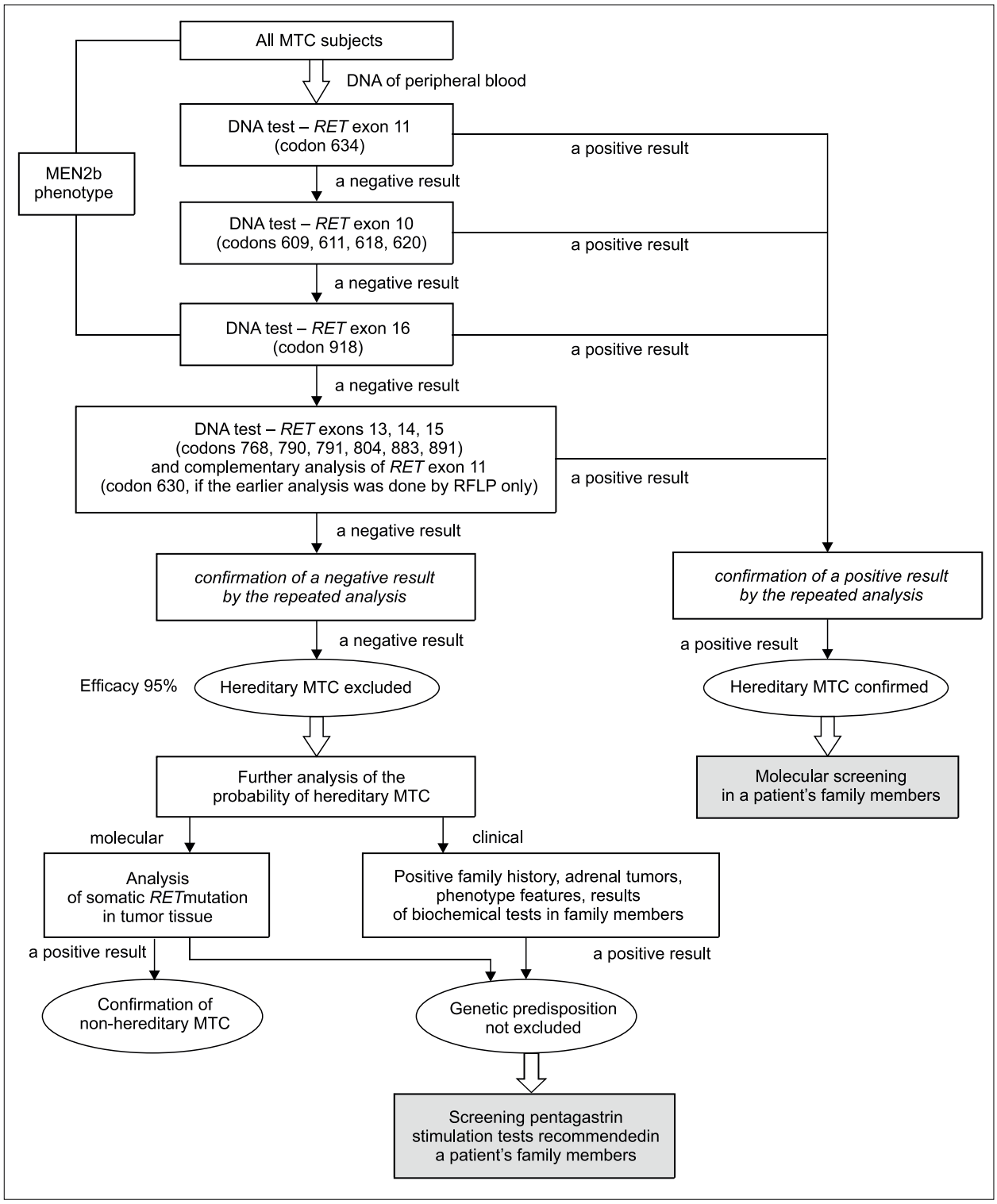

Fig. 3. The algorithm of DNA testing in MTC patients.

A negative result of *RET* mutation analysis is equally important as it allows to exclude a patient from the further follow up if the mutation, characteristic for a particular family, is not detected. However, a negative result obtained after MTC diagnosis has a predictive value of 90% (2). There are some families (mostly with FMTC) in whom, despite a few MTC cases, the *RET* protooncogene mutation has never been confirmed. Thus, if a family history is positive and a DNA test is negative, even after whole gene sequencing, the only solution is to continue yearly pentagastrin stimulation tests in the whole family.

BIOCHEMICAL TESTS IN DIAGNOSTICS AND FOLLOW-UP OF MTC AND MEN 2 SYNDROME

MTC cells secrete large amounts of calcitonin. Its measurement enables MTC diagnosis preoperatively and the assessment of treatment effectiveness and

disease follow-up (22-24). Currently, routine pentagastrin stimulation tests in family members are no longer necessary as hereditary form of MTC is detected by a DNA analysis. Calcitonin measurement should be carried out in a specialized lab. The reference values of sensitive tests are below 10 or several pg/ml. Calcitonin assessment is crucial for the proper evaluation of MTC treatment efficacy. Normalization of preoperatively elevated serum calcitonin level after the surgery confirms its curative character. Persistent hypercalcitoninemia in spite of radical operation at both macroscopic and microscopic levels speaks for the presence of MTC microfoci in regional lymph nodes or in the liver.

A low calcitonin level (below 10-12 pg/ml) in the subsequent assessments, performed every 3 months, confirm MTC complete remission. However, a calcitonin stimulation test should be performed once a year. Intravenous calcium or pentagastrin administration, or oral omeprazole are used to stimulate calcitonin secretion by MTC cells. The most popular is pentagastrin test. Serum calcitonin level is measured 2, 5 and 10 minutes after pentagastrin infusion (0.5 μg/kg). An increase in serum calcitonin level above 30 pg/ml suggests the presence of residual MTC cells. A high basal calcitonin concentration indicates the necessity of additional radiological assessment to localize the disease.

A normal calcitonin level in patients with disseminated MTC is rarely observed. In some patients it may be a consequence of poorly differentiation of cancer and the lack of its hormonal activity. It should be remembered, that a high antigen concentration (of calcitonin) may inhibit its binding to antibodies used in a radioimmunological assay what leads to a false negative result. To avoid this phenomenon serum sample need to be diluted. Serum dilution is often necessary because calcitonin levels in disseminated MTC are very high as they may exceed the range of measurable concentrations even 10-1000 times. Never tests are free of Hook effect.

The other MTC markers are less important. The most popular one is carcinoembryonic antigen. An increase in serum CEA level reflects highly advanced MTC.

A biochemical diagnosis of pheochromocytoma requires the assessment of serum level of catecholamines and/or their methoxy derivatives urinary excretion (25).

Serum calcium evaluation and native PTH measurement (by immunoradiometric assay) are used to diagnose hyperparathyroidism.

IMAGING STUDIES

Apart from classical radiological examinations, very helpful for MTC and pheochromocytoma diagnosis, scintigraphic imaging with radiopharmaceuticals selectively used for MTC or pheochromocytoma such as: ^{123}I-mIBG, antiCEA or anticalcitonin radiolabelled monoclonal antibodies and radiolabelled somatostatin analogs also plays a leading role. In the recent years ^{18}FDG-PET/CT and ^{18}F-DOPA PET/CT have become much more important. However, in many cases is still not possible to localize cancer foci despite an elevated serum calcitonin level. Calcitonin is a very sensitive cancer marker, which points out persistent MTC before it is detectable by imaging studies.

Both neck US and 99mTc-MIBI subtractive scintigraphy are used in diagnostics of hyperparathyroidism. These examinations are quite successful to localize parathyroid adenoma but not to confirm parathyroid hyperplasia, which is a common reason of hyperparathyroidism in MEN 2a syndrome.

TREATMENT OF HEREDITARY MTC

The treatment of clinically overt hereditary and sporadic MTC does not differ significantly. However, some issues characteristic for a hereditary form need to be considered (11). The most important is to exclude pheochromocytoma before thyroid surgery. The other ones are wider indications for the elective neck lymphadenectomy (performed regardless whether lymph node metastases are present or not but with consideration of basal serum calcitonin level).

The Polish recommendations concerning MTC surgical treatment were prepared in 1995 and subsequently modified in 2000, 2006 and 2010 (32), whereas the guidelines devoted to pheochromocytoma were published in 2006 (27).

Surgical treatment

Radical thyroid operation is the treatment of choice in MTC patients. The minimal extent of surgery involves total thyroidectomy with concomitant central neck lymph node dissection. Many authors believe that lateral neck lymphadenectomy should also be routinely performed in all subjects, while a less extensive approach may be considered only in young patients (12).

Multifocal tumor growth and a high risk of lymph node metastases accompanied by high serum calcitonin concentration justify much wider than in differentiated thyroid cancer indications for elective neck lymphadenectomy.

At least in 50% of patients presents lymph node metastases at diagnosis. Resection of upper mediastinal lymph nodes via neck approach is recommended in patients with central neck lymph node metastases. However, in the recent years personalized approach based on a type of RET mutation, patient's age and serum calcitonin concentration is rather considered (28).

In a case of pheochromocytoma laparoscopic surgery with subtotal resection of adrenal gland is the treatment of choice. The most important issue is to prepare the patient properly by at least 2-week α-adrenergic receptor antagonists administration (25).

Surgery also constitutes the main therapeutic strategy in hyperparathyroidism. Usually radical parathyroidectomy (the resection of 3 and 1/2 of all parathyroid glands) is necessary as parathyroid hyperplasia is present in most patients. Nevertheless, disease recurrences are quite common. Therefore, pharmaco-

therapy based on biphosphonates or calcimimetics is considered as the first line treatment to keep surgical approach only for these subjects in whom pharmacological treatment is not successful.

Radiotherapy

Benefits of complementary postoperative radiotherapy have not been unequivocally proved. Therefore it is not routinely recommended.

Chemotherapy

Both mono- and polychemotherapy are not successful in MTC treatment.

Tyrosine kinase inhibitors

Several phase II and III studies proved the efficacy of VEGFR and RET inhibitors in the treatment of advanced, progressive MTC. Currently vandetanib and cabozantinib are registered for advanced and progressive MTC both in the US and Europe.

^{131}I-mIBG therapy

There are no indications to radioiodine ^{131}I treatment in MTC. However, ^{131}I-mIBG used to be administered in advanced MTC. Moreover, due to TKIs registration ^{131}I-mIBG therapy is not longer recommended in MTC but it is still administered in patients with disseminated or inoperative pheochromocytoma.

MANAGEMENT RECOMMENDED IN ASYMPTOMATIC RET PROTOONCOGENE MUTATION CARRIERS

The development of molecular diagnostics arises a question what therapeutic consequences should be associated with the detection of mutation carriers in apparently healthy family members. The current guidelines are presented in table 3.

Hereditary MTC is a type of cancer where prophylactic surgery is beneficial. The first announcement with reference to prophylactic thyroidectomy occurred in 1995. Wells et al. reported 5 family members, diagnosed with MEN 2a syndrome in whom detection of the *RET* protooncogene mutation resulted in total thyroidectomy despite normal basal and pentagastrin stimulated serum calcitonin levels. In two analogous subjects, described by Pacini et al., multifocal MTC was diagnosed. The discussion, published in Surgery in 1995, underlined that the question related to prophylactic surgery was not "whether to operate" but "when to operate" (22).

The analysis of published data concerning prophylactic thyroidectomy demonstrates that in *RET* exon 10 and 11 mutation carriers with negative pentagastrin stimulate test the risk of MTC microfoci detection is 35-40%, whereas ~ 55% of subjects show C-cell hyperplasia. Only in 10% of patients the postoperative histopathological examination is normal. When pentagastrin stimulation test is positive MTC microfoci are diagnosed in up to 85% of subjects.

These results are confirmed by our own data as in all children, operated in our center, c-cell hyperplasia was present. In 3 children between 7 and 12 year of age the time when pentagastrin stimulation test started to become positive was caught (21). C-cell hyperplasia, revealed by microscopic examination, precedes positive result of pentagastrin stimulation test – the minimal c-cell volume is crucial to cause an increased in calcitonin secretion, even in stimulation tests.

Table 3. Management recommended in MTC patient's family members.

	FMTC	MEN 2A	MEN 2B
DNA test	As soon as possible after RET mutation detection of RET mutation in family member with clinically overt MTC, the optimal time:		
	Up to 6 year of life	2-3 year of life	Just after the birth
Basal serum calcitonin assessment	In all family members simultaneously with DNA test		
Pentagastrin stimulation test	In these RET mutation carriers in whom basal calcitonin is normal; Every year in these RET mutation carriers who refused prophylactic thyroid surgery		
Neck US	Just after RET mutation detection, next every year		
Prophylactic thyroidectomy	At the age of 5-6	At the age of 5-6	Up to 1st year of life
metoxycatecholamines in daily urine collection	Not necessary in codon 768 and 891 RET mutation carriers. When other mutations are present the assessment is necessary in these families in whom at least 4 family members have MTC without any case of pheochromocytoma	At the age of 5, and then, after the age of 10 every year	At the age of 2, and then every year
CT scan of adrenal glands	Not necessary in codon 768 and 891 RET mutation carriers. When other mutations are present the assessment is necessary in these families in whom at least 4 family members have MTC without any case of pheochromocytoma	First time at the age of 10, next every2-3 years	First time at the age of 5, and then after the age of 10 every year
Serum calcium and PTH assessment	Not recommended	Between 20 and 30 year of age every 2 years and then every year	Not recommended

These results were summed up by Włoch in his habilitation thesis (28). Presenting the benefits of prophylactic surgery it should be emphasized that the risk of MTC onset before 35-40 year of age exceeds 95% when the *RET* protooncogene mutation is confirmed. In most *RET* mutation carriers MTC develops between 10 and 15 year of age, however the earlier onset is also possible. Moreover, according to the published data, the risk of unsuccessful prophylactic surgery is ~ 50% if the operation is carried out when pentagastrin stimulation test is positive or even when basal calcitonin is increased. Despite its macroscopic radical character postoperative calcitonin remains elevated.

The reason of an unsuccessful surgery is related to multifocal tumor growth and early lymph node metastases. The prophylactic surgery may be considered as beneficial long-term clinical outcomes (more than 90% patients cured) may be achieved only in an early cancer stage as well as the risk of postoperative complication does not exceed 2%, only if the thyroid surgery is performed in an experienced clinical center. Moreover, L-thyroxin (LT4) administration is easy to follow-up and guarantee normal child development if LT4 substitutive doses are adequate and keep a child euthyreotic. Therefore, a fear of hypothyroidism and its clinical consequences does not justify postponing surgery.

The opponents of a prophylactic surgery believe that repeated stimulated calcitonin measurements make both an early MTC diagnosis and early surgery possible. Thus, it reduces the risk of an unnecessary operation. However, on the basis of the own experience C-cell hyperplasia was present in all patients, even when pentagastrin stimulation test was normal. According to the published data such approach unnecessarily increases the risk of cancer progression, especially that any possible advantages of postponed surgery are rather small with simultaneous significant risk of MTC progression.

Dralle et al. emphasize that prophylactic thyroidectomy should involve central neck lymph node dissection when serum calcitonin concentration is abnormal or a patient is older than 10 years (28). They call this procedure “pre-emptive surgery”. Bilateral neck lymphadenectomy is recommended when lymph node metastases are present or when serum calcitonin concentration is abnormal or a patient is older than 15 years (29, 30).

Separate issues are long-term complications related to total thyroidectomy. According to the most authors they are rare as a study, reported by Dralle at al. assessed the risk of postoperative hypoparathyroidism and unilateral laryngeal recurrent nerve injury as 6.7% 1.3% respectively. This risk was not dependent on child’s age.

The most important criterion supporting prophylactic thyroidectomy is the increase of treatment benefits – the higher percentage of cured patients without any bigger risk of treatment complications. However, because of prophylactic thyroidectomies have been performed since 1994, there are still no long-term outcomes. Nevertheless, in the vast majority of patients, also on the basis of our own experience, serum calcitonin normalization was achieved (28). So far, no case of recurrent hypercalcitoninemia after prophylactic surgery was reported. Although, it may theoretically happen, also because of the presence of C-cells in thymus. Due to too short follow-up the issue related to pheochromocytoma development in patients after prophylactic thyroidectomy is still an opened question. To date no recommendation with reference to prophylactic adrenalectomy exists.

CONCLUSIONS

The issues to keep in mind:

1. Each MTC case requires DNA test to exclude a germinal RET protooncogene mutation, even if family history is negative.
2. Screening for the RET protooncogene mutation in family members of all subjects with hereditary MTC is obligatory because it facilitates the early MTC and other possible MEN 2 endocrinopathies diagnosis.
3. Prophylactic thyroidectomy is recommended in asymptomatic RET mutation carriers.
4. A lot of cases of hereditary MTC are related to RET mutations causing MEN 2a syndrome, where MTC may be accompanied by pheochromocytoma and (much more rarely) hyperparathyroidism. Therefore, it is recommended to check whether a particular type of the RET mutation requires screening for pheochromocytoma and primary hyperparathyroidism.
5. In a classical MEN 2a syndrome the operation of pheochromocytoma should precede total thyroidectomy due to MTC.
6. In a patient with MEN 2a syndrome not only incorrectly treated MTC but also incorrectly treated pheochromocytoma may result in death.

BIBLIOGRAPHY

1. Hazard JB, Hawk WA, Crile G Jr: Medullary (solid) carcinoma of the thyroid – a clinicopathology entity. J Clin Endocrinol Metab 1959; 19: 152-161.
2. Eng C: RET proto-oncogene in the development of human cancer. J Clin Oncol 1999; 17(1): 380-393.
3. Wiench M, Włoch J, Wygoda Z et al.: Genetic diagnosis of multiple endocrine neoplasia type 2B. Endokrynologia Polska 2000; 51: 67-76.
4. Barbot N, Calmettes C, Schuffenecker I et al.: Pentagastrin stimulation test and early diagnosis of medullary thyroid carcinoma using immunoradiometric assay of calcitonin: comparison with genetic screening in hereditary medullary thyroid carcinoma. J Clin Endocrinol Metab 1994; 78: 114-120.
5. Donis-Keller H, Dou S, Chi D et al.: Mutations of the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 1993; 2: 851-856.
6. Hofstra RM, Landsvater RM, Ceccherini I et al.: A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 1994; 367: 375-376.

7. Mulligan LM, Kwok JBJ, Healey CS et al.: Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993; 363: 458-460.
8. Eng C, Clayton D, Schuffenecker I et al.: The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET Mutation Consortium analysis. JAMA 1996; 276: 1575-1579.
9. Eng C, Mulligan LM: Mutations of the RET proto-oncogene in the multiple endocrine neoplasia type 2 syndromes, related sporadic tumors, and Hirschsprung disease. Human Mutation 1997; 9: 97-109.
10. Gagel RF, Cote GJ: Pathogenesis of medullary thyroid carcinoma. Thyroid Cancer. Kluwer Academic Publisher. Boston/Dordrecht/ London 1998.
11. Brandi ML, Gagel RF, Angeli A et al.: Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab 2001; 86: 5658-5671.
12. Gimm O, Sutter T, Dralle H: Diagnosis and therapy of sporadic and familial medullary thyroid carcinoma. J Cancer Res Clin Oncol 2001; 127: 156-165.
13. Kitamura Y, Goodfellow PJ, Shimizu K et al.: Novel germline RET protooncogene mutations associated with medullary thyroid carcinoma (MTC): mutation analysis in Japanese patients with MTC. Oncogene 1997; 14: 3103-3106.
14. Berndt I, Reuter M, Saller B et al.: A new hot spot for mutations in the RET protooncogene causing familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2A. J Clin Endocrinol Metab 1998; 83: 770-774.
15. Bolino A, Schuffenecker I, Luo Y et al.: RET mutations in exons 13 and 14 of FMTC patients. Oncogene 1995; 10: 2415-2419.
15. Eng C, Smith DP, Mulligan LM et al.: A novel point mutation in the tyrosine kinase domain of the RET proto-oncogene in sporadic medullary thyroid carcinoma and in family with FMTC. Oncogene 1995; 10: 509-513.
16. Hofstra RM, Fattoruso O, Quadro L et al.: A novel point mutation in the intracellular domain of the RET protooncogene in a family with medullary thyroid carcinoma. J Clin Endocrinol Metab 1997; 82: 4176-4178.
17. Wohllk N, Cote GJ, Bohalho MMJ et al.: Relevance of RET proto-oncogene mutations in sporadic medullary thyroid carcinoma. J Clin Endocrinol Metab 1996; 81: 3740-3745.
18. Jarząb B, Włoch J, Wiench M et al.: Wczesna diagnostyka zespołu mnogiej gruczolakowatości wewnątrzwydzielniczej typu 2 poprzez analizę genetyczną germinalnych mutacji protoonkogenu RET. Endokrynol Pol 1999; 50: 127-134.
19. Lips CJ, Landsvater RM, Hoppener J et al.: Clinical screening as compared with DNA analysis in families with multiple endocrine neoplasia type 2A. N Eng J Med 1994; 331: 828-835.
20. Włoch J, Wygoda Z, Wiench M et al.: Profilaktyczne całkowite wycięcie tarczycy u nosicieli mutacji w protoonkogenie RET powodujących dziedzicznego raka rdzeniastego tarczycy. Pol Przegl Chir 2001; 73: 569-585.
21. Krassowski J, Słowińska-Srzednicka J, Gietka M et al.: Oznaczanie kalcytoniny w rozpoznawaniu i ocenie wyników leczenia raka rdzeniastego tarczycy. Pol Tyg Lek 1989; 44: 757-759.
22. Pacini F, Fontanelli M, Fugazzola L et al.: Routine measurement of serum calcitonin in nodular thyroid diseases allows the preoperative diagnosis of unsuspected sporadic medullary thyroid carcinoma. J Clin Endocrinol Metab 1994; 78: 826-829.
23. Wasylewski A, Skrzypek J, Kokot F, Śledziński Z: Przydatność oznaczania kalcytoniny w ocenie doszczętności zabiegu operacyjnego u chorych z rakiem rdzeniastym tarczycy. Endokrynol Pol 1981; 32: 239-244.
24. Januszewicz A: Nadciśnienie tętnicze. Zarys patogenezy, diagnostyki i leczenia. Medycyna Praktyczna, Kraków 2002.
25. Rekomendacje Diagnostyka i Leczenie raka tarczycy przyjęte podczas III Konferencji naukowej. Rak tarczycy, Szczyrk, 25.03.2006. Endokrynol Pol 2006; 57: 458-477.
26. Januszewicz A, Januszewicz W, Jarząb B, Więcek A: Wytyczne dotyczące diagnostyki i leczenia chorych z guzem chromochłonnym. Nadciśnienie tętnicze 2006; 10: 1-19.
27. Włoch J: Postępowanie chirurgiczne w dziedzicznym raku rdzeniastym tarczycy: modyfikacje wynikające z diagnostyki mutacji germinalnych protoonkogenu RET i badania profilu molekularnego guzów (rozprawa habilitacyjna). Nowotwory 2007; 57(5): 1-51.
28. Dralle H, Gimm O, Simon D et al.: Prophylactic thyreoidectomy in 75 children an adolescent with hereditary medullary thyroid carcinoma: German and Austrian experience. World J Surg 1998, 22: 744-750.
29. Skinner M, Moley JA, Dilley WG et al.: Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. N Engl J Med 2005; 15(353): 1105-1113.
30. Baudin E, Travagli JP, Schlumberger M: How effective is prophylactic thyroidectomy in asymptomatic multiple endocrine neoplasia type 2A? Nat Clin Pract Endocrinol Metab 2006, 2: 256-257.
31. Bałdys-Waligórska A, Barczyński M, Bręborowicz D et al.: Diagnostyka i leczenie raka tarczycy – rekomendacje polskie. Endokrynol Pol 2010; 5(61): 518-568.
32. Krajewska J, Jarzab B: Lenvatinib for the treatment of radioiodine-refractory follicular and papillary thyroid cancer. Expert Opinion on Orphan Drugs 09/2014; DOI: 10.1517/21678707.2014.962514.
33. Krajewska J, Jarzab B: Novel therapies for thyroid cancer Expert Opinon on Pharmacother. Expert Opin Pharmacother 2014 Dec; 15(18): 2641-2652.

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