Tamara Janić[1], Mirjana Stojković[1,2], Sanja Klet[1], Bojan Marković[1], Biljana Nedeljković Beleslin[1,2], Jasmina Ćirić[1,2], Miloš Žarković[1,2]

AGRESIVNI KLINIČKI TOK MEDULARNOG MIKROKARCINOMA ŠTITASTE ŽLEZDE

Sažetak: Medularni karcinom štitaste žlezde je oblik neuroendokrinog tumora koji nastaje iz parafolikularnih C-ćelija koje proizvode kalcitonin. Pored kalcitonina, ove ćelije produkuju i manje količine drugih peptide, među kojima je i karcinoembrionalni antigen (CEA) koji se koristi kao nespecifični tumor marker u praćenju pacijenata sa ovim tumorom. MTC je redak tumor štitaste žlezde i javlja se tri puta češće kod žena nego kod muškaraca. Može se javiti u dva oblika, sporadičan (80%) i familijarni oblik (20%). Familijarni oblik se može javiti samostalno ili udružen sa drugim endokrinim tumorima u sklopu sindroma MEN 2A i MEN 2B. Sporadična forma se najčešće javlja u petoj i šestoj deceniji života. Familijarni oblik se nasleđuje autozomno dominantno, najčešće u osnovi leži mutacija RET protoonkogena lociranog na 10. hromozomu. Premalignom lezijom smatra se hiperplazija C ćelija, koja prethodi medularnom karcinomu. Medularni karcinom daje metastaze veoma rano. Prikazali smo pacijentkinju sa sporadičnom formom MTC koja se javila u tipičnom životnom dobu. Inicijalne vrednosti i baznog i stimulisanog kalcitonina nisu bile u opsegu za sumnju na MCT, ali je zbog perzistentnog porasta kalcitonina, uz povišen bazni (63 pg/mL) i viši stimulisani kalcitonin (96 pg/mL), pacijentkinja upućena na operativno lečenje. S obzirom na jaku korelaciju vrednosti kalcitonina sa veličinom tumora, očekivano su inicijalne vrednosti kalcitonina bile niske jer je tumor bio veličine 3 mm. Promena na štitastoj žlezdi okarakterisana je kao hiperplazija C-ćelija. Međutim, s obzirom na činjenicu da je nodularnu C-ćelijsku hiperplaziju histopatološki teško razlikovati od medularnog mikrokarcinoma, na osnovu perzistentnog porasta vrednosti kalcitonina kod pacijentkinje je već u vreme tiroidektomije najverovatnije postojala metastatska bolest (tada je viđen suspektan LN u jugularnom lancu desno). Definitivna dijagnoza je postavljena tek biopsijom promene u jetri. Uvedena je terapija tirozin kinaznim inhibitorima, na kojima se beleži

[1] Klinika za endokrinologiju, dijabetes i bolesti metabolizma, Univerzitetski klinički centar Srbije

[2] Medicinski fakultet Univerziteta u Beogradu

pad vrednosti kalcitonina, ali porast karcinoembrionalnog antigena, koji je loš prognostički parametar.

Ključne reči: Medularni karcinom štitaste žlezde, hiperplazija C-ćelija, agresivan tok bolesti, metastaze, kalcijumski test, tirozin kinazni inhibitori, kalcitonin, karcinoembrionalni antigen

Prikaz slučaja

Pacijentkinja, 56 godina, primljena je na Kliniku za endokrinologiju, dijabetes i bolesti metabolizma UKCS zbog metastatskog medularnog karcinoma štitaste žlezde. Prvi put se javila endokrinologu novembra 2018. g. zbog mikronodusa u štitastoj žlezdi, kada je izmeren kalcitonin 4,4 ng/L. Jula 2019. god. kontrolni kalcitonin granično viši, 8,1 ng/L, zbog čega je urađen kalcijumski test koji nije pokazao prekomerni skok kalcitonina (Tabela 1). U daljem toku se registruje porast baznog kalcitonina (Tabela 1). Marta 2020. godine EHO štitaste žlezde opisuje dve do tri mikrociste u distalnom delu desnog lobusa (DL), kao i hipoehogen mikronodus 3mm. U srednjem delu DL posteriorno nepravilan izo- do heteroehogen nodus sa nepravilnim, zadebljalim, hipoehogenim haloom bez CD signala, promera 5x5x6 mm. U levom lobusu (LL) nekoliko mikrocisti. U srednjem delu jugularnog lanca desno uvećan, hipoehogen limfni nodus (LN) promera 5x9 mm sa centralnom vaskularizacijom, ne može se sa sigurnošću reći da li se radi o reaktivnom ili patološki izmenjenom LN. TIRADS DL 4 LL 2. Oktobra 2020. god. učinjena je iglena biopsija nodusa u DL, CP nalaz: u analiziranom punktatu nalazi se mala količina koloida, brojni eritrociti, umereno brojni limfociti, oskudan crtasti ćelijski debri, malobrojne pojedinačne i grupisane folikulske ćelije. Bethesda II. Vrednosti serumskog kalcijuma i fosfata, kao i metaboliti kateholamina u 24h urinu uredni (Tabela 1).

Zbog porasta kalcitonina indikovano je operativno lečenje, a 30. 12. 2020. učinjena totalna tiroidektomija. Intraoperativno, na preseku preparata nodus u desnom lobusu promera oko 3 mm, parenhimske strukture. PH nalaz: DL: **Hiperplazija C-ćelija i papilarni mikrokarcinom.** U postoperativnom periodu se beleži dalji rast kalcitonina (Tabela 1). Hospitalizovana maja 2021. kada su učinjena vizualizaciona ispitivanja: MR abdomena i CT pluća pokazali su multiple metastatske promene **u jetri i plućima,** MR kičmenog stuba nije pokazao sekundarne depozite. Tokom ove hospitalizacije učinjena je biopsija promena u jetri: PH dg: carcinoma medullare glandulae thyreoideae metastaticum in hepate. Imunohistohemijski (IHH) nalaz: Calcitonin (+), Synaptophysin (+), mCEA (+) i Thyreoglobulin (-). Pacijentkinja upućena na procenu za stereotaksičnu radioterapiju (STRT) meta promena na jetri i na plućima, ali je zaključeno da STRT nije indikovana zbog broja meta promena. **Scintigrafija somatostatinskih receptora** (^{99m}Tc-Tektrotyd): Nakupljanje radiofarmaka u opisanim promenama uz segmentne bronhe za donji režanj, spikuliranoj

promeni anterobazalnom segmentu desno i pojedinim hipodenzim promenama u jetri ukazuje na diskretnu ekspresiju somatostatinskih receptora (Krenning score 1). **CT endokranijuma sa kontrastom** nije pokazao jasne znake ekspanzivnih promena. Započeta je procedura za terapiju tirozin kinaznim inhibitorima.

Reevaluacija stanja pacijentkinje učinjena je januara 2022. Rezultati laboratorijskih analiza prikazani su u Tabeli 2. **EKG:** sin. ritam, fr 88/min, PQ interval 0.16s, bez promena ST segmenta i T talasa.. **EHO vrata:** Štitasta žlezda je operisana. Lokalno nalaz uredan. U proksimalnom delu jugularnog lanca desno (nivo II) vidi se suspektan LN promera 4x11 mm sa suspektnim mikrokalcifikatima. U distalnom delu jugularnog lanca levo vide se dva patološki izmenjena LN, veći je promera 9x13 mm. U distalnom delu jugularnog lanca desno vide se dva patološki izmenjena LN, veći je promera 3x5 mm. **CT grudnog koša:** U anterobazalnom segmentu donjeg desnog plućnog režnja prisutna je infiltrativna centralno nekrotična promena dimenzija 41x30x20 mm, sa kalcifikatima centralno, koja je širokom osnovom u kontaktu sa interlobarnom i dijafragmalnom pleurom. Hilarno desno nekrotične patološki izmenjene limfne žlezde dijametra do 12 mm, traheobronhijalno desno do 13 mm, paratrahealno desno do 12 mm. Traheja i bronhijalno stablo su normalnog dijametra i grananja, bez uočljivih zadebljanja zida i intraluminalnog sadržaja. Prikazane medijastinalne strukture su normalne prezentacije. Nema pleuralnog izliva. Pršljensko telo Th6 je sa ovalnom zonom osteoskleroze dijametra 20 mm. Jetra je u svim segmentima zahvaćena hipodenznim nejasno ograničenim promenama sa hiperdenznim rubom izgleda sekundarnih depozita. Nadbubrežne žlezde su normalne morfologije, bez nodularnih zadebljanja. **MRI abdmena: n**a bazalnim presecima kroz grudni koš uočava se mekotkivna promena u mediobazalnom segmentu donjeg desnog lobusa. Jetra je uredne veličine, sa multiplim prethodno verifikovanim promenama restriktivne difuzije, MR karakteristika sekundarnih depozita, najveći u IIS/IIIS više konfluentnih ukupnog dijametra 72x43 mm, u IS dijametra 27x27 mm. Prosta cista promera 7 mm u IIIS, kao i nekoliko mikrocisti. Žučna kesa je elongirana, distendirana dijametra 75x45 mm (KKx LL), sa gušćim staznim sedimentom na dnu promera do 22 mm. Uočavaju se multipli solitarni LN, najveći hepatogastrično promera do 10 mm. Ne vidi se ascit. Ostali CT nalaz uredan. **MR Th kičme:** Nalaz odgovara degenerativnim promenama bez sekundarnih depozita. **Kraniogram:** Na kostima krova lobanje ne vide se patološke promene. Februara 2022. uvedena terapija tirozin kinaznim inhibitorom (vandetanib), nakon čega se beleži značajan pad kalcitonina, ali i porast CEA (Tabela 1).

Diskusija

Medularni karcinom štitaste žlezde (MCT) je oblik neuroendokrinog tumora koji nastaje iz parafolikularnih C-ćelija, izvedenih iz neuralnog grebena, koje proizvode kalcitonin. Pored kalcitonina, ove ćelije produkuju i manje količine drugih peptide,

među kojima je i karcinoembrionalni antigen (CEA) koji se koristi kao nespecifičan tumor marker u praćenju pacijenata sa ovim tumorom. MTC obuhvata 3–12% svih tiroidnih karcinoma i javlja se tri puta češće kod žena nego kod muškaraca (Ž : M=3 : 1). (1) Može se javiti u dva oblika, sporadičan (80%) i familijarni oblik (20%). Familijarni oblik se može javiti samostalno ili udružen sa drugim endokrinim tumorima u sklopu sindroma MEN 2A (pored medularnog karcinoma, obuhvata i primarni hiperparatiroidizam i feohromocitom) i MEN 2B (obuhvata feohromacitom, mukozne ganglioneurome i mafanoidni habitus). Sporadična forma se najčešće javlja u petoj i šestoj deceniji života. Nasledna forma se može javiti u bilo kom uzrastu, najčešće u mlađem, dok se izolovani oblik javlja obično u petoj deceniji života. Familijarni oblik se nasleđuje autozomno dominantno, najčešće u osnovi leži mutacija RET protoonkogena lociranog na 10. hromozomu. Sporadični oblik medularnog karcinoma ima lošiju prognozu od familijarnog oblika u MEN2 sindromu, a tumor u sklopu MEN 2B sindroma pokazuje najveću biološku agresivnost.

Premalignom lezijom smatra se hiperplazija C-ćelija, koja prethodi medularnom karcinomu. Mnogo je češća u naslednom obliku, ali se javlja i u nekim sporadičnim slučajevima MCT. Postoje dve forme C-ćelijske hiperplazije koje imaju jasno različite patogenetske mehanizme, fiziološka ili reaktivna C-ćelijska hiperplazija i neoplastična C-ćelijska hiperplazija koja predstavlja karcinom in situ. (2,3) Kada neoplastična C-ćelijska hiperplazija pokazuje dominantno nodularan tip rasta teško ju je razlikovati od medularnog mikrokarcinoma, pri čemu je ova diferencijacija od izuzetnog značaja s obzirom na metastatski potencijal mikrokarcinoma. (4) U familijarnom obliku MCT tumori su češće multicentrični, bilateralni, multitipni i obično manji u vreme postavljanja dijagnoze, dok je sporadični oblik najčešće jednostran. Histološki se zapažaju brojne varijante, ali histološki tip medularnog karcinoma nema značaja za prognozu bolesti. Pored histoloških karakteristika, za postavljanje dijagnoze neophodna je IHH analiza, a bojenja se vrše na tumor-specifični marker – kalcitonin i panneuroendokrine markere – hromogranin (CgA), neurospecifična enolaza (NSE) i sinaptofizin. Kalcitonin se dokazuje imunocitohemijski u 95–100% MCT, a CEA u 77–100%. CEA je senzitivan, ali ne i specifičan za medularni karcinom. Tumori sa slabijom pozitivnošću na kalcitonin imaju agresivnije biološko ponašanje. (5,6) Medularni karcinom, pored kalcitonina, luči i druge biološke markere: CEA, CgA, CGRP (calcitonin gene related peptid), NSE, serotonin, ACTH, vimentin, citokeratin, glucagon, pri čemu je CEA dobar prognostički marker. Nivo kalcitonina korelira sa veličinom tumora. Postoperativno, nivo kalcitonina ukazuje na rezidualno tkivo i služi kao parametar praćenja eventualne progresije bolesti. MTC se širi limfogeno u vrat i medijastinum i hematogeno kada daje udaljene metastaze u plućima, kostima i jetri. Medularni karcinom daje metastaze veoma rano. Metastaze u limfnim čvorovima su prisutne u do 43% MTC $\leq$ 10 mm, od kojih je 20% neizlečivo. (7)

Dijagnostički postupci kod sumnje na MCT, nakon vizuelizacije nodusa, primarno su usmereni na detekciju proizvoda tumora, odnosno kalcitonina kao glavnog biološkog markera. Rutinsko merenje serumskog kalcitonina može da otkrije hiperplaziju C-ćelija ili medularni karcinom. Ipak, uredne vrednosti kalcitonina ne isključuju tumor C-ćelija. U slučaju povišenih baznih vrednosti kalcitonina radi se stimulacioni test kalcijumom. (8, 9) Kod nekonkluzivnog ili suspektnog nalaza iglene biopsije štitaste žlezde na MTC savetuje se IHH ispitivanje ispirka igle na kalcitonin, CgA i CEA uz dokaz odsustva tiroglobulina. (10)

Rutinsko merenje serumskog kalcitonina kod pacijenata sa nodularnom/multinodularnom strumom predstavlja najbolji metod za ranu identifikaciju nesumnjivog medularnog karcinoma. Pokazano je da su pacijenti kod kojih je rutinski određivan kalcitonin imali manje uznapredovali stadijum MTC, a u postoperativnom toku češću normalizaciju serumskog kalcitonina i bolju dugoročnu prognozu u poređenju sa pacijentima bez rutinskog određivanja kalcitonina. Razlog za ovakav ishod je postavljena dijagnoza MTC u ranoj fazi, što je od suštinskog značaja u lečenju ovog karcinoma. (11) Indikacije za rutinsko merenje kalcitonina još uvek nisu univerzalno predložene od strane naučnih društava. ETA je 2006. godine preporučila merenje kalcitonina u početnoj obradi tiroidnih nodusa (12), novije ATA i AACE/ACE/AME smernice su neodređene po pitanju rutinskog merenja kalcitonina (ne preporučuju ni za ni protiv). (13, 14) Ipak, kod pacijenata kod kojih je MCT dijagnostikovan i lečen u kasnijoj fazi potrebna su kontrolna merenja kalcitonina tokom celog života, skupe dijagnostičke obrade zbog rezidualne bolesti ili dodatne hirurške procedure, te bi troškovi ovih dodatnih testova i reoperacija mogli biti znatno veći od onih za otkrivanje jednog slučaja u ranoj fazi. Nedovoljna senzitivnost i specifičnost merenja kalcitonina i dalje dovodi u pitanje vrednost njegove rutinske upotrebe zbog niske prevalencije medularnog karcinoma štitaste žlezde (0,32% kod pacijenata sa nodularnim oboljenjem štitne žlezde). Nedostatak rutinskog merenja kalcitonina je velika varijabilnost vrednosti kalcitonina u zavisnosti od laboratorije/vrste testa (15,16), kao i nalaz nešto viših bazalnih nivoa kalcitonina od normalnog opsega zbog drugih uzroka. Zato je u interpretaciji rezultata neophodno isključiti ostale moguće uzroke povišenih vrednosti kalcitonina (primena inhibitora protonske pumpe, hronična bubrežna insuficijencija, pseudohipopituirizam, ektopična proizvodnja kalcitonina od strane netiroidnih neuroendokrinih tumora, hipergastrinemija, hronični tiroiditis, interferencija sa heterofilnim antitelima…). U radu Fugazzola i saradnika iz 2020. godine predstavljene su granične vrednosti baznog kalcitonina koje potencijalno mogu da ukažu na MTC, a to su vrednosti kalcitonina veće od 30 ng/L za žene i veće od 34 ng/L za muškarce, dok su najstroži pragovi za kalcijumom stimulisani kalcitonin bili > 79 ng/L za žene i >466 ng/L za muškarce. Pokazalo se da bazni kalcitonin ima visoku tačnost, iako su neki slučajevi dijagnostikovani samo testom stimulacije. Kombinovanjem vrednosti baznog kalcitonina, koji je bilo ispod ili iznad graničnih vrednosti

sa stimulisanim kalcitoninom iznad graničnih vrednosti, svi slučajevi MTC bili su ispravno identifikovani. Medijana i srednja vrednost bili su 21,38 i 15 ng/L (opseg 2,8–53,7) za tumore <5 mm, 52,26 i 58,8 ng/L (opseg 5,6–126) za tumore 5–10 mm, 227,6 ng/L (opseg 12,9–1860) za tumore ≥ 10 mm ($P<0,001$). Pronađena je značajna korelacija između veličine tumora i nivoa baznog kalcitonina. Sa druge strane, nije bilo značajne korelacije između veličine tumora i nivoa stimulisanog kalcitonina. (17) ATA smernice za lečenje MCT iz 2015. godine (10) savetuju totalnu tiroidektomiju, disekciju cervikalnih LN u zavisnosti od seroloških, vizualizacionih i intraoperativnih nalaza. Radioterapija eksternim snopom (EBRT) se primenjuje na vrat ako postoje dokazi o ekstenzivnoj lokalnoj bolesti, rezidualnoj bolesti ili ekstratiroidnom širenju. Poznato je da MCT, kao neuroendokrini tumor, eksprimira somatostatinske receptore (SSTR), te terapija radionuklidima peptidnih receptora može imati i dijagnostičku i terapijsku vrednost. Terapija tirozin-kinaznim inhibitorima (TKI) ima svoje mesto kod pacijenata sa progresivnom simptomatskom metastatskom bolešću. TKI su inhibitori malih molekula koji specifično ciljaju i inhibiraju delovanje tirozin kinaza. Pošto je RET protoonkogen oblik receptora tirozin kinaze, TKI mogu da inhibiraju fosforilaciju RET proteina, što dovodi do regulacije njegovih nishodnih reakcija i posledične inhibicije rasta tumora. Danas se koristi više TKI: imatinib, gefitinib, motesanib, sunitinib, sorafenib, aksitinib, apatinib, pazopanib, lenvatinib, vandetanib i kabozantinib. Uspešno se može koristiti i lokalna krio-, termo- ili hemo-ablacija metastaza u jetri. (18, 19)

Prikazali smo pacijentkinju sa sporadičnom formom MTC koji se javio u tipičnom životnom dobu. Inicijalne vrednosti i baznog i stimulisanog kalcitonina nisu bile u opsegu za sumnju na MCT, ali je zbog perzistentnog porasta kalcitonina, uz povišen bazni (63 pg/mL) i viši stimulisani kalcitonin (96 pg/mL), pacijentkinja upućena na operativno lečenje. S obzirom na korelaciju vrednosti kalcitonina sa veličinom tumora, očekivano su inicijalne vrednosti kalcitonina bile niske jer je tumor bio veličine 3 mm. Promena na štitastoj žlezdi okarakterisana je kao hiperplazija C-ćelija, što predstavlja premalignu leziju, koja je ređa u sporadičnoj formi bolesti, a revizijom PH nalaza nije nađen kriterijum za preinačenje dijagnoze u medularni karcinom štitaste žlezde. Međutim, s obzirom na činjenicu da je nodularnu C-ćelijsku hiperplaziju histopatološki teško razlikovati od medularnog mikrokarcinoma, na osnovu perzistentnog porasta vrednosti kalcitonina, najverovatnije je da je kod pacijentkinje već u vreme tiroidektomije postojala metastatska bolest (tada je viđen suspektan LN u jugularnom lancu desno). Definitivna dijagnoza je postavljena tek biopsijom promene u jetri. S obzirom na raširenost bolesti, nije bila indikovana STRT meta promena te je pacijentkinji nastavljeno lečenje tirozin-kinaznim inhibitorom na kom se registruje pad vrednosti kalcitonina, ali uz istovremeni porast CEA, što je nepovoljan pokazatelj toka bolesti.

Tabela 1.

	Kalcitonin (ng/L)			
2018. g.	4,4 ng/L			
2019. g. jul	8,1 ng/L – granično viši			
Kalcijumski test 2019. g.	9,5…33,6…32,7…31,2…26,9…25,7 ng/L			
2019. g. decembar	13,2 ng/L			
2020. g. januar	22,4 ng/L			
2020. g. februar	25,2 ng/L			
Kalcijumski test 2020. g. novembar	63 ng/L…96 ng/L			
2020. g. decembar	84 ng/L sa razblaženjem 1:10 – 94,8 ng/L, RIA U punktatu 85,8 ng/L (RIA), 92,1 ng/L			metanefrin 0,44 umol/24h normetanefrin 2,0 umol/24h
2020. g. decembar	**Tireoidektomija**			
2021. g. april	151 ng/L			
2021. g. maj	303,4 ng/L	362 ng/L		**CEA**(μg/L)
2021. g. avgust	1167 ng/L			20,9 μg/L
2022. g. januar	7789 ng/L			216,0 μg/L
2022. g. februar	**Uvedena terapija vandetanibom**			
2022. g. mart	7559 ng/L			473,0 μg/L
2022. g. maj	2000 ng/L			497,5 μg/L
2022. g. jun	>2000 ng/L			915,3 μg/L

Tabela 2.

RBC	5.14	Glc	4.4	Albumini	39	gama-GT	176	PO4	1.0
HGB	140	Urea	5.1	HOL	4.67	LDH	516	Mg	0.79
HTC	0.409	Kreatinin	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 ukupni	6.8	Tg	1.54	K	2.8	PTH	< 3.0
PLT	424	Bilirubin direktan	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 %	Proteini	68	ALP	258	Ca++	1.48	fT4	16.8

Literatura

1. Hundahl SA, Cady B, Cunningham MP, et al. Initial results from a prospective cohort study of 5583 cases of thyroid carcinoma treated in the united states during 1996. US and German Thyroid Cancer Study Group An American College of Surgeons Commission on Cancer Patient Care Evaluation study Cancer. 2000; 89: 202–217.
2. Perry A, Molberg K, Albores-Saavedra J. Physiologic versus neoplastic C-cell hyperplasia of the thyroid: separation of distinct histologic and biologic entities. Cancer 1996; 77: 750–756.
3. Albores-Saavedra, J., Krueger, J.E. C-Cell hyperplasia and medullary thyroid microcarcinoma. Endocr Pathol 12, 365–377 (2001). https://doi.org/10.1385/EP:12:4:365
4. McDermott MB, Swanson PE, Wick MR. Immunostains for collagen type IV discriminate between C-cell hyperplasia and microscopic medullary carcinoma in multiple endocrine neoplasia, type 2a. Hum Pathol 26: 1308–1312, 1995.
5. Das DK, Mallik MK, George SS, Sheikh ZA, Pathan SK, Haji BE, Al-Quaddomi SA, Mirza K, Ajrawi MT, Amir T, Dey P, Francis IM. Secretory activity in medullary thyroid carcinoma: A cytomorphological and immunocytochemical study. Diagn Cytopathol. 2007 Jun; 35(6): 329–37.
6. Bockhorn M, Sheu SY, Frilling A, Molmenti E, Schmid, KW, Broelsch CE.: Paraganglioma-like medullary thyroid carcinoma: a rare entity. Thyroid. 2005 Dec; 15 (12): 1363–7.
7. Machens A, Henning Dralle H. Biological relevance of medullary thyroid microcarcinoma. J Clin Endocrinol Metab. 2012; 97: 1547–1553. doi: 10.1210/jc.2011-2534.
8. Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iacconi P, Basolo F, Pinchera A, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab. 2004; 89: 163–168. doi: 10.1210/jc.2003-030550.
9. Sand M, Gelos M, Sand D, Bechara FG, Bonhag G, Welsing E, Mann B: Serum calcitonin negative Medullary thyroid carcinoma. World Journal of Surgical Oncology 2006, 4: 97.
10. Samuel A. Wells, Sylvia L. Asa, Henning Dralle, Rossella Elisei, Douglas B. Evans, Robert F. Gagel, Nancy Lee, Andreas Machens, Jeffrey F. Moley, Furio Pacini, Friedhelm Raue, Karin Frank-Raue, Bruce Robinson, M. Sara Rosenthal, Massimo Santoro, Martin Schlumberger, Manisha Shah, and Steven G. Waguespack. Revised American Thyroid Association Guidelines for the Management of Medullary Thyroid Carcinoma. American Thyroid Association. Volume 25, Number 6, 2015. doi: 10.1089/thy.2014.0335.
11. Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iacconi P, Basolo F, Pinchera A, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab. 2004; 89: 163–168. doi: 10.1210/jc.2003-030550.

12. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W, Taskforce ETC. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol. 2006; 154: 787–803. doi: 10.1530/eje.1.02158.
13. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016; 26: 1–133. doi: 10.1089/thy.2015.0020.
14. Gharib H, Papini E, Garber JR, Duick DS, Harrell RM, Hegedüs L, Paschke R, Valcavi R, Vitti P, AACE, ACE, AME Task Force on Thyroid Nodules American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules–2016 update. Endocr Pract. 2016; 22: 622–639. doi: 10.4158/EP161208.GL.
15. Wells SA, Jr, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moley JF, Pacini F, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015; 25: 567–610. doi: 10.1089/thy.2014.0335.
16. Trimboli P, Giovanella L, Crescenzi A, Romanelli F, Valabrega S, Spriano G, Cremonini N, Guglielmi R, Papini E. Medullary thyroid cancer diagnosis: an appraisal. Head Neck. 2014; 36: 1216–1223. doi: 10.1002/hed.23449.
17. L. Fugazzola,M. Di Stefano, S. Censi,A. Repaci, C. Colombo, F. Grimaldi, F. Magri, U. Pagotto, M. Iacobone, L. Persani,and C. MianBasal and stimulated calcitonin for the diagnosis of medullary thyroid cancer: updated thresholds and safety assessment. J Endocrinol Invest 2021; 44(3): 587–597. doi: 10.1007/s40618-020-01356-9.
18. Katherine A. Araque , Sriram Gubbi , Joanna Klubo-Gwiezdzinsk. Updates on the Management of Thyroid Cancer, Horm Metab Res 2020; 52(8): 562–577. doi: 10.1055/a-1089-7870.
19. Mijin Kim, Bo Hyun Kim. Current Guidelines for Management of Medullary Thyroid Carcinoma. Endocrinology and Metabolism. 2021; 36(3): 514-524. doi: https://doi.org/10.3803/EnM.2021.1082.

Tamara Janić[1], Mirjana Stojković[1,2], Sanja Klet[1], Bojan Marković[1], Biljana Nedeljković Beleslin[1,2], Jasmina Ćirić[1,2], Miloš Žarković[1,2]

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.

[1] Clinic of Endocrinology, Diabetes and Metabolic Disease, University Clinical Center of Serbia

[2] Faculty of medicine, University of Belgrade

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

References

1. Hundahl SA, Cady B, Cunningham MP, et al. Initial results from a prospective cohort study of 5583 cases of thyroid carcinoma treated in the united states during 1996. US and German Thyroid Cancer Study Group An American College of Surgeons Commission on Cancer Patient Care Evaluation study Cancer. 2000; 89: 202–217.
2. Perry A, Molberg K, Albores-Saavedra J. Physiologic versus neoplastic C-cell hyperplasia of the thyroid: separation of distinct histologic and biologic entities. Cancer 1996; 77: 750–756.
3. Albores-Saavedra, J., Krueger, J. E. C-Cell hyperplasia and medullary thyroid microcarcinoma. Endocr Pathol 12, 365–377 (2001). https://doi.org/10.1385/EP:12:4:365
4. McDermott MB, Swanson PE, Wick MR. Immunostains for collagen type IV discriminate between C-cell hyperplasia and microscopic medullary carcinoma in multiple endocrine neoplasia, type 2a. Hum Pathol 26: 1308–1312, 1995.
5. Das DK, Mallik MK, George SS, Sheikh ZA, Pathan SK, Haji BE, Al-Quaddomi SA, Mirza K, Ajrawi MT, Amir T, Dey P, Francis IM. Secretory activity in medullary thyroid carcinoma: A cytomorphological and immunocytochemical study. Diagn Cytopathol. 2007 Jun; 35(6): 329–37.
6. Bockhorn M, Sheu SY, Frilling A, Molmenti E, Schmid, KW, Broelsch CE.: Paraganglioma-like medullary thyroid carcinoma: a rare entity. Thyroid. 2005 Dec; 15 (12): 1363–7.
7. Machens A, Henning Dralle H. Biological relevance of medullary thyroid microcarcinoma. J Clin Endocrinol Metab. 2012; 97: 1547–1553. doi: 10.1210/jc.2011-2534.
8. Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iacconi P, Basolo F, Pinchera A, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab. 2004; 89: 163–168. doi: 10.1210/jc.2003-030550.
9. Sand M, Gelos M, Sand D, Bechara FG, Bonhag G, Welsing E, Mann B: Serum calcitonin negative Medullary thyroid carcinoma. World Journal of Surgical Oncology 2006, 4: 97.
10. Samuel A. Wells, Sylvia L. Asa, Henning Dralle, Rossella Elisei, Douglas B. Evans, Robert F. Gagel, Nancy Lee, Andreas Machens, Jeffrey F. Moley, Furio Pacini, Friedhelm Raue, Karin Frank-Raue, Bruce Robinson, M. Sara Rosenthal, Massimo Santoro, Martin Schlumberger, Manisha Shah, and Steven G. Waguespack. Revised American Thyroid Association Guidelines for the Management of Medullary Thyroid Carcinoma. American Thyroid Association. Volume 25, Number 6, 2015. doi: 10.1089/thy.2014.0335
11. Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iacconi P, Basolo F, Pinchera A, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab. 2004; 89: 163–168. doi: 10.1210/jc.2003-030550.

12. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W, Taskforce ETC. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol. 2006; 154: 787–803. doi: 10.1530/eje.1.02158.
13. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, et al. 2015. American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016; 26: 1–133. doi: 10.1089/thy.2015.0020.
14. Gharib H, Papini E, Garber JR, Duick DS, Harrell RM, Hegedüs L, Paschke R, Valcavi R, Vitti P, AACE, ACE, AME Task Force on Thyroid Nodules American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules–2016 update. Endocr Pract. 2016; 22: 622–639. doi: 10.4158/EP161208.GL.
15. Wells SA, Jr, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moley JF, Pacini F, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015; 25: 567–610. doi: 10.1089/thy.2014.0335.
16. Trimboli P, Giovanella L, Crescenzi A, Romanelli F, Valabrega S, Spriano G, Cremonini N, Guglielmi R, Papini E. Medullary thyroid cancer diagnosis: an appraisal. Head Neck. 2014; 36: 1216–1223. doi: 10.1002/hed.23449.
17. L. Fugazzola,M. Di Stefano, S. Censi,A. Repaci, C. Colombo, F. Grimaldi, F. Magri, U. Pagotto, M. Iacobone, L. Persani,and C. MianBasal and stimulated calcitonin for the diagnosis of medullary thyroid cancer: updated thresholds and safety assessment. J Endocrinol Invest 2021; 44(3): 587–597. doi: 10.1007/s40618-020-01356-9
18. Katherine A. Araque , Sriram Gubbi , Joanna Klubo-Gwiezdzinsk. Updates on the Management of Thyroid Cancer, Horm Metab Res 2020; 52(8): 562–577. doi: 10.1055/a-1089-7870
19. Mijin Kim, Bo Hyun Kim. Current Guidelines for Management of Medullary Thyroid Carcinoma. Endocrinology and Metabolism. 2021; 36(3): 514-524. doi: https://doi.org/10.3803/EnM.2021.1082