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ORIGINAL ARTICLE

Intraoperative calcitonin stimulation testing in the surgical treatment of C-cell disease

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Key words: **medullary thyroid cancer (MTC); C-cell hyperplasia (CCH); surgery; calcitonin; intraoperative calcitonin stimulation testing (iCST)**

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Abstract

OBJECTIVE: The prognosis of medullary thyroid carcinoma (MTC), derived from parafollicular C-cells, depends on the completeness of the initial surgical excision. The C-cells produce calcitonin, a peptide hormone used as a biochemical and immunohistochemical tumor marker. The aim of the study was to evaluate an individualized approach to patients with C-cell disease, i.e. MTC and C-cell hyperplasia (CCH), using the intraoperative calcitonin testing-assisted surgical strategy as a predictor of the final outcome.

STUDY DESIGN: A unicentre cross-sectional study.

Methods: From June 2009 to May 2015, thirty one patients with MTC/CCH were surgically treated primarily (n=24) or reoperated for persistence of the disease (n=7). Depending on the result of intraoperative calcitonin stimulation testing (iCST), patients underwent total thyroidectomy with or without lymph node dissection. All patients were tested repeatedly in the postoperative period (range 1 to 48 months).

RESULTS: The iCST was true negative in all CCH, and ten out of eleven N0 MTC primarily operated patients, and true positive in one N0 patient and six of the seven reoperated patients. The test was false negative in two patients preoperatively evaluated as N+, one primarily operated and one reoperated, respectively.

CONCLUSION: The results encourage the use of an individualised approach on patients with MTC/CCH, e.g. to be less radical surgically in cases of negative iCST, and to be more radical in those patients with persistent increase of serum calcitonin. The absence of post-stimulation calcitonin elevation in iCST seems to be a good prognosis indicator in patients with an early-stage C-cell disease, but longer follow-up is needed.

Abbreviations:

CCH	- C-cell hyperplasia
CEA	- carcinoembryonic antigen
FNAB	- fine-needle aspiration biopsy
hCT	- human calcitonin
iCST	- intraoperative calcitonin stimulation test
iHP	- intraoperative histopathology
LN	- lymp node
MTC	- medullary thyroid carcinoma
USG	- ultrasonography

INTRODUCTION

Medullary thyroid carcinoma (MTC) is a tumor originating from parafollicular C-cells that produce calcitonin, a peptide hormone which is involved in calcium homeostasis (Kebebew 2000). Calcitonin is used as a very efficient biochemical and immunohistochemical tumor marker. Calcitonin is released from C-cells and tumor cells after stimulation by various provocative agents (Lamari 1996). This fact enables us to use calcitonin testing in routine preoperative diagnosis and postoperative follow-up. While a negative postoperative result in calcitonin measurement ("biochemical remission") predicts definitive cure in a high percentage of patients, postoperative increase in the basal or stimulated calcitonin level is an unfavorable prognostic factor, implying disease recurrence or persistence (Kebebew 2000).

Surgical treatment is the only modality that can possibly lead to a complete cure of the patient. The importance of the initial operation, including total thyroidectomy and lymph node dissection of adjusting metastases including micrometastases, is indisputable. Patients with local lymph node metastases have significantly impaired long-term survival when compared to early-stage diagnosed patients (Kebebew 2000; Van Heerden 1990). The purpose of surgery is to avoid overresection at the initial operation, but remembering the potential complications that occur more frequently after reoperations. Subsequent surgery is frequently required when an unexpected unsatisfactory result is encountered in patients initially treated in surgical centers not familiar with the surgical management of patients with MTC.

To date, it is not possible to predict whether the tumor has been completely removed after surgery. Therefore, it is necessary to develop novel strategies to get more information about the persistence of the tumor tissue intraoperatively. The aim of the present study was to evaluate an individualized approach to patients with C-cell disease, using the intraoperative calcitonin testing (iCST)-assisted surgical strategy as a predictor of the final outcome after surgery.

MATERIALS AND METHODS

Patients

Thirty one patients (16 women and 15 men, mean age 57,9 years) underwent surgical treatment with iCST, from June 2009 to May 2015. All patients were treated at the Department of Otolaryngology/Head & Neck Surgery, Central Military Hospital and Faculty Hospital in Ružomberok, Slovakia. Primary surgery was performed in 24 patients (P1–P24). Revision surgery for persistence of MTC was performed in 7 patients (R1–R7). These 7 patients were initially treated in different surgical centers.

Surgical protocol

All surgical procedures were performed using optical magnification and a harmonic scalpel with the surgical method as follows. After total thyroidectomy, both lobes of the thyroid gland were intraoperatively evaluated by histopathologic examination (iHP) (see below). A) In cases of suspected CCH without evidence of MTC, total thyroidectomy alone was performed. B) In cases of MTC proven by iHP without any preoperative radiological findings of metastases (N0), total thyroidectomy, bilateral central node dissection, and ipsilateral lymph node dissection (level III–IV), followed by iCST was performed. According to the results of the stimulation test we either continued or skipped contralateral comprehensive lymph node dissection. Since an interim analysis of the first four patients (see Results), we have changed the surgical method in patients assessed preoperatively as N0 to a less radical approach, when dissection of the lateral compartments on the side of the tumor was omitted in cases with negative iCST(see Discussion). C) In cases of MTC proven by iHP with preoperative radiological findings of lymph node metastases (N+), total thyroidectomy, bilateral central node dissection, and comprehensive lymph node dissection (level II–V) on the side of the tumor, followed by iCST was performed. Depending on the result of iCST, we either continued or skipped contralateral comprehensive lymph node dissection (level II–V). D) In cases of revision surgery for suspicious lymph node metastases and/or biochemical persistance of the disease, we performed iCST after the resection of suspicious compartments and in cases of subsequent positive results of the stimulation test, we continued with yet unresected compartments.

Intraoperative calcitonin stimulation testing (iCST) and evaluation of clinical outcome

After finishing the resection, calcium gluconate was administered intravenously (2 mg/kg of body weight) with blood sampling at 0, 1, 2, and 5 minute. The blood sampling site was an antecubital vein. We considered the absence of post-stimulation increment as a „negative result". The persistence of poststimulation increase of calcitonin was considered a „positive result". The increments in calcitonin levels were expressed as relative increase in calcitonin and calculated as the ratio of peak calcitonin level after stimulation divided by the respective basal calcitonin level. At least a 1.2-fold (20%) increase was considered as clinically significant. Four follow-up outcomes of the iCST were defined:

1. True positive: the iCST came up positive and calcitonin was still elevated in postoperative follow-up. 2. False positive: the iCST came up positive but calcitonin normalized postoperatively. 3. True negative: the iCST came up negative and calcitonin normalized postoperatively. 4. False negative: the iCST came up negative but calcitonin was elevated in postoperative follow-up.

Calcitonin assay

From June 2009 to September 2010, serum calcitonin was measured by a one-step sandwich chemiluminiscence immunoassay (CLIA) from the DiaSorin LIAISON® Calcitonin Assay (DiaSorin, MN, USA) on LIAISON® Analyzer. From October 2010 until the end of study, serum calcitonin was measured by a second-generation CLIA assay (LIAISON® Calcitonin II-Gen). Both assays measure between 1 pg/mL and 2000 pg/mL (1 pg/mL = 0.29 pmol/L). The lowest reportable value is 1 pg/mL. Values below 1 pg/mL are reported as <1 pg/mL. The highest reportable value without dilution is 2000 pg/mL. Any sample higher than the reportable range is reported as >2000 pg/mL. In some patients, samples were diluted in the DiaSorin LIAISON Calcitonin Specimen Diluent or LIAISON Calcitonin II-Gen Specimen Diluent respectively, re-assayed and recalculated.

Pathological examination

All samples were assessed by the same pathologist specializing in thyroid gland pathology without prior knowledge of the clinical history. The intraoperative biopsy (iHP) of both lobes of the thyroid was performed as a routine part of the management of the patient with suspicion of CCH/MTC. The iHP was evaluated using standard hematoxylin-eosin-based histopathologic examination of frozen sections. Postoperatively, both lobes of the thyroid gland were evaluated. The thyroid gland was fixed in formalin, then horizontally sliced, and each section was embedded in paraffin. Serial 4-µm sections of each block were used for routine stains (hematoxylin-eosin-saffron) and immunohistochemistry. Deparaffinized sections of all blocks were stained for CT (polyclonal rabbit anti-CT serum at 1/1000 dilution; DAKO SA), thyroglobulin, synaptophysin, chromogranin A, carcinoembryonic antigen, neuro-specific enolase (DAKO), and evaluated as recommended previously (Kasserer 2002).

RESULTS

Primarily operated patients for MTC

Fifteen patients (P1–P15) were operated on the suspicion of MTC. In two cases (P1,P9), the diagnosis of nodular goiter was established only intraoperatively by iHP, without preoperative fine-needle apiration biopsy (FNAB) nor calcitonin evaluation. FNAB was performed in ten patients, and was suggestive of MTC in eight of them (P2, P3, P5, P7, P8, P12, P14, P15), of malignancy in one case (P4) and negative in one case (P10). We did not perform FNAB in three patients (P6, P11, P13), in whom serum calcitonin levels were highly suggestive of MTC. Twelve patients (P1–P10, P12, P15) had no radiological evidence of metastases (N0) preoperatively, one patient (P6) was radiologically N+. All patients underwent total thyroidectomy. Bilateral central node dissection and comprehensive ipsilateral lymph node dissection was performed in seven patients (P1–P4, P11, P13, P14). Bilateral central node dissection only was performed in eight patients (P5–P10, P12, P15) (Table 1).

The iCST was negative in twelve patients. During postoperative follow-up (1 to 36 months), nine N0 and two N+ (P10, P12) were without clinical, biochemical or radiological evidence of reccurence. Only one N+ patient (P11) had slightly increased postoperative basal and post-stimulation calcitonin levels (Table 2) without radiological evidence of tumor persistence or recurrence.

The iCST was positive in three patients (P13–P15). In one N0 patient (P14) and two N+ patients (P13, P15) disease persisted biochemically in the postoperative follow-up (5–20 months).

Patients reoperated for persistent MTC

Seven patients (R1–R7) underwent revision surgery for residual MTC (Table 1). In patient R1, after total thyroidectomy and ipsilateral central compartment dissection, we performed a revision of the thyroid bed with revisional lymph node dissection ipsilateraly (levels II–V), including the dissection of an involved jugulary vein. In patient R2, after total thyroidectomy, we performed revisional lymph node dissection in the central and lateral compartments (levels II–VI). In patient R3, after total thyroidectomy and bilateral central compartment dissection, revisional lymph node dissection of the ipsilateral cervical compartments was done (levels II–V). In patient R4, an incomplete lateral neck dissection of metastases from an unknown primary tumor, that has shown to be MTC, was done at the initial neck surgery. We performed total thyroidectomy, bilateral central compartment dissection and ipsilateral revisional neck dissection (levels II–V). In patient R5, after total thyroidectomy and lymphadenectomy of unknown extension, a bilateral central compartment dissection and ipsilateral neck dissection (levels II–V) was performed. In patient R6, after total thyroidectomy we performed a revision of the thyroid bed, bilateral central compartment dissection and ipsilateral neck dissection (levels II–V). In patient R7, after total thyroidectomy and after revisional neck dissection of unknown extension with 1 positive lymph node, we performed a revision of the thyroid beds, bilateral central compartment dissection and ipsilateral neck dissection (levels II–V).

The iCST was positive in six (R1–R3, R5–R7) out of seven patients. In five patients (R1–R5) we noted a decrease of basal calcitonin, with persistence of its

Tab. 1. Preoperative, intraoperative and postoperative clinical evaluation, extension of dissection and lymph node involvement.

n	Age	Sex	Preoperative		Intraoperative		Postoperative		LN metastases		
			USG LN+	FNAB	Dissection	iHP	Histology	Tumor stage	Ipsilateral	Contralateral	Outcome
P1	76	F	no	n.d.	III,IV,**VI**	MTC	MTC	T1N0M0	Negative	negative	remission
P2	59	M	no	MTC/CCH†	III,IV,**VI**	MTC	MTC	T1N0M0	Negative	negative	remission
P3	58	F	no	MTC/CCH†	III,IV,**VI**	MTC	MTC	T1N0M0	Negative	negative	remission
P4	49	F	no	malignant	III,IV,**VI**	MTC	MTC	T1N0M0	Negative	negative	remission
P5	51	F	no	MTC	VI	MTC	MTC	T2N0M0	Negative	negative	remission
P6	47	M	no	n.d.	VI.	MTC	MTC	T1N0M0	negative	negative	remission
P7	64	M	no	MTC	VI.	MTC	MTC	T1N0M0	negative	negative	remission
P8	56	F	no	MTC	VI.	MTC	MTC	T1N0M0	negative	negative	remission
P9	61	F	no	n.d.	VI.	MTC	MTC	T1N0M0	negative	negative	remission
P10	81	F	no	Negative	VI.	MTC	MTC	T2N0M0	negative	negative	remission
P11	35	F	yes	n.d.	II,III,IV,V,**VI**	MTC, LN+	MTC	T3N1bM0	II,IV,V,VI	negative	persistence
P12	58	F	no	MTC	VI.	MTC	MTC	T3N1aMO	VI.	negative	remission
P13	37	M	yes	n.d.	II,III,IV,V,**VI**	MTC	MTC	T1N1bMO	III,IV,VI	positive	persistence
P14	60	F	yes	MTC	III,IV,**VI**	MTC	MTC	T1N0M0	negative	negative	persistence
P15	67	M	no	MTC	VI	MTC	MTC	T1N1aM0	VI	VI	persistence
P16	57	F	no	negative	n.d.	negative	CCH	NA	NA	NA	remission
P17	38	M	no	n.d.	n.d.	negative	CCH	NA	NA	NA	remission
P18	59	F	no	n.d.	n.d.	negative	CCH	NA	NA	NA	remission
P19	35	M	no	negative	n.d.	CCH	CCH	NA	NA	NA	remission
P20	45	F	no		n.d.	negative	CCH	NA	NA	NA	remission
P21	61	F	no	negative	n.d.	n.d.	CCH	NA	NA	NA	remission
P22	44	F	no	negative	n.d.	negative	CCH	NA	NA	NA	remission
P23	51	M	no	MTC/CCH†	n.d.	negative	CCH	NA	NA	NA	remission
P24	63	M	no	n.d.	n.d.	negative	CCH	NA	NA	NA	remission
R1	64	M	yes	mts LN	II,III,IV,V,VI#	n.d.	MTC	T3N1bM0	II,III,IV	NA	persistence
R2	64	M	no	negative	II,III,IV,V,VI	LN+	MTC	T3N1bM0	III,VI	negative	persistence
R3	70	F	yes	n.d.	II,III,IV,V ‡	n.d.	MTC	T3N1bM1	II,III,IV,V	NA	persistence
R4	50	M	yes	mts LN	I,II,III,IV,V,**VI**	MTC, LN+	MTC	T2N1bM1	II,IV,V,VI	negative	persistence
R5	63	M	no	n.d	II,III,IV,V,**VI**	n.d.	MTC	T2N1bM0	II,VI	Negative	persistence
R6	53	M	yes	MTC	II,III,IV,V,**VI**	MTC	MTC	T2N1bM0	III,IV,VI	negative	persistence
R7	63	M	yes	n.d.	II,III,IV,V,**VI**	n.d.	MTC	T1N1bM0	II,VI	negative	persistence

Abbreviations: bold, bilateral dissection; CCH, C-cell hyperplasia; FNAB, fine-needle aspiration biopsy; iHP, intraoperative histopathology (frozen sections); LN+, metastatic lymph node(s); LN- lymph node(s) without metastates; MTC, medullary thyroid cancer; mts LN, lymph node(s) with metastatic medullary thyroid cancer; NA, not applicable; n.d., not done; USG LN+, suspected lymph node(s) at ultrasonography; †, FNAB in patients P2, P4 and P23 was suggestive to MTC/CCH due to high calcitonin levels in wash-out fluid from fine-needle aspirates; #, ipsilateral dissection of central compartment (level VI) was done at initial surgery; ‡, bilateral dissection of central compartment (level VI) was done at initial surgery

poststimulation increase during iCST in four (R1–R3, R5) out of five patients. In one patient (P6) basal calcitonin level didn't change and in one patient (P7) even increased with poststimulation increase during iCST in both cases.

In postoperative follow-up (22–48 months) the disease persisted biochemically in all patients, without radiologic evidence in six cases (R1, R2, R4–R7). In patient R3 postoperative FDG-PET revealed the persistence of mediastinal metastases.

Patients with C-cell hyperplasia (CCH)

The group with CCH consisted of nine patients (P16–P24). Preoperative calcitonin values were established in all of them (Table 2). All patients underwent total thyroidectomy with iHP of both lobes except one (P2). The iHP was positive for CCH in one case only, and iCST was negative in all patients. In definitive histological evaluation, linear and nodular CCH was found in all cases. In initial follow-up (2 to 42 months), calcitonin levels are suggestive of biochemical remission in all patients.

Tab. 2. Results of preoperative, intraoperative and postoperative basal and peak calcitonin levels in stimulation testing.

n	Age	Sex	Preoperative		Intraoperative		Postoperative		Follow-up	
			hCT_0	hCT_{max}	hCT_0	hCT_{max}	hCT_0	hCT_{max}		
P1	76	F	n.d.	n.d.	157	159	<1	<2	18	m
P2	59	M	327	>2,000	97	98	<1	<1	21	m
P3	58	F	96	5,080	19	19	2	2	12	m
P4	49	F	853	>2,000	432	437	<1	3	9	m
P5	51	F	1,699	3,100	310	356	<1	<1	11	m
P6	47	M	191	n.d.	87	175	<2	<2	1	m
P7	64	M	569	n.d.	218	216	<2	<2	1	m
P8	56	F	1169	101840	448	530	<2	<2	4	m
P9	61	F	n.d.	n.d.	120	116	<2	<2	36	m
P10	81	F	n.d.	n.d.	2000	1981	2	8	2	m
P11	35	F	12,400	25,000	4,210	4,190	13	105	5	m
P12	58	F	6790	n.d.	>2,000	1756	<2	<2	30	m
P13	37	M	2803	n.d.	1560	4860	616	947	20	m
P14	60	F	189	2785	74	178	2,46	48,8	6	m
P15	67	M	182	822	271	790	152	487	5	m
P16	57	F	15	825	19	18	<1	<1	14	m
P17	38	M	36	n.d.	11	11	<1	<1	20	m
P18	59	F	12	506	14	17	<1	<1	15	m
P19	35	M	33	514	19	18	<1	<1	14	m
P20	45	F	16	1085	8	8	<2	<2	42	m
P21	61	F	40	582	11	14	<1	<1	12	m
P22	44	F	16	504	13	13	<1	<1	12	m
P23	51	M	16	166	1	1	<2	<2	6	m
P24	63	M	17	1010	14	11	<1	<1	2	m
R1	64	M	361	1,890	298	449	89	171	22	m
R2	64	M	178	1,173	136	465	122	>2,000	22	m
R3	70	F	>2,000	>2,000	148	>2,000	140	>2,000	27	m
R4	50	M	444	>2,000	287	277	3836	6685	48	m
R5	63	M	53	531	478	891	4673	4802	32	m
R6	53	M	13	1084	13	37	5	331	41	m
R7	63	M	53	531	249	365	5634	11561	34	m

Abbreviations: hCT0, basal calcitonin; hCTmax, peak calcitonin; m, months; not done; P1–P24, primary operated patients; R1–R7, reoperated patients

DISCUSSION

MTC exists as a sporadic or hereditary form. Several germline mutations in the *RET* proto-oncogene are the source of diferent phenotypes including familial MTC (FMTC) and multiple endocrine neoplasia 2A (MEN 2A) and MEN 2B. C-cell hyperplasia, or rather its pathologic variation, is connected with hereditary MTC. It can be considered as a preneoplastic condition that may procede to MTC within a variable period of time depending on the genotype (Hinze 2001).

Surgical treatment is currently the only modality that can offer complete cure of MTC, though patients with bilateral node involvement should be considered to have a systemic disease and unlikely to be biochemically cured even following surgical treatment (Machens 2007a). Thus, in MTC patients with suspected limited local metastatic disease, total thyroidectomy with bilateral central neck dissection (level VI) in radiologically negative and ipsilateral or bilateral modified neck dissection (levels II–V) for clinically or radiologically identifiable disease, is currently recommended (Kloos 2009).

Tumor size (more than 10 mm) significantly correlates with the presence of lymph node metastases, as already demonstrated in many series (Tamagnini

2005). Among others, multifocal tumor growth, intraoperative evidence of thyroid capsule infiltration (Mikkoli 2007), presence of desmoplastic stromal reaction (Scheuba 2006), increased carcinoembryonic antigen (CEA) levels (>30 ng/mL) (Machens 2007b) and high level of basal and poststimulation calcitonin (more than 10-fold increase) (Machens 2008), have been identified as different predictors of metastases in MTC in an individual approach. Ideally, such predictors should provide information about the persistence of the tumor tissue intraoperatively.

Although the measurement of basal calcitonin is a highly sensitive method for the detection of MTC, it presents a low specificity for this tumor. Several physiologic and pathologic conditions other than C-cell disease have been associated with increased levels of calcitonin (Lamari 1996; Toledo 2009).

As mentioned before, human calcitonin is a well-accepted marker of C-cell proliferation. Both basal and stimulated levels of calcitonin are widely used in the preoperative management and also in postoperative follow-up (Kebebew 2000; VanHeerden 1990). Postoperative calcitonin level normalization in MTC patients can occur during variable periods. Brauckhoff *et al.* (2001) investigated the postoperative elimination kinetics of calcitonin in patients who were operated on for MTC or CCH. In patients suffering from CCH, the basal calcitonin was not detectable within 3 hours postoperatively with the elimination half-life of calcitonin less than 1 hour. In patients suffering from MTC the elimination half-life of calcitonin ranged from 4 to 24 hours. As other studies have failed to establish the interrelation between basal calcitonin normalization and preoperative calcitonin values (Scheuba 2007), the longer elimination half-time of calcitonin in cases of MTC comparing to CCH reflects residual disease or an intraoperative dissemination (Brauckhoff 2001).

The iCST was introduced by Scheuba *et al.* (2007) , but the application of the procedure in clinical practice has been sporadic. Current rapid chemiluminescent assays allow the iCST to be used more commonly, especially in surgical centers specializing in thyroid cancer. Short calcitonin assay run time enables the results to be obtained in approximately 40–45 minutes and allows the continuation of surgery as required. Faggiano *et al.* (2012) recently evaluated intraoperative basal calcitonin level 30 minutes post surgical resection in patients with an early-stage C-cell disease. In their study, a decrease of basal calcitonin of less than 50 percent 30 minutes after thyroid surgery suggested incomplete C-cell tumor tissue removal. Keeping in mind that the timing of sampling was different in our study, using this criterion, 7 out of 17 patients with true negative result of iCST in our study would have been false positive and *vice versa*. 1 patient with biochemical persistence would be erroneously assessed as cured. Therefore, basal calcitonin is not useful in intraoperative evaluation of the completeness of tumor tissue removal according to our results. De Crea in his study also showed that intraoperative basal calcitonin is not highly accurate in predicting the completness of surgical resection. Specificity, sensitivity and accuracy were 78,2, 66,6 and 76,9%, respectively (De Crea 2014).

It has been estimated that the calcitonin serum level is a good measure of tumor volume, but total C-cell mass multiplied by the cells mean rates of calcitonin secretion more accurately account for serum calcitonin levels (Machens 2008). The synthetic analog gastrin pentapeptide (pentagastrin), or calcium gluconate are widely used for biochemical diagnosis of primary or recurrent MTC. Even in MTC patients, rates of calcitonin secretion may differ in localized and advanced tumors because of the loss or dysfunction of the CCK-B/gastrin receptor during de-differentiation (Behr 2002). The absence of CCK-B/gastrin receptor expression seems to account for negative pentagastrin stimulation tests. There are occasionally patients with poorly differentiated MTC associated with limited calcitonin levels despite large tumor deposits. The C-cells of the thyroid are known to express the calcium-sensing receptor (CaSR). Freichel *et al.* (1996) provided substantial evidence, both molecular and functional, for the presence of CaSR in the human C-cell carcinoma cell line. Thus, this receptor contributes to the stimulation of calcitonin secretion in C-cells, and allows us to use calcium gluconate as an attractive alternative to pentagastrin in iCST. To the best of our knowledge, there are no studies evaluating the loss or dysfunction of CaSR during de-differentiation of MTC.

The price to be paid for the prospects of cure and protection from neck invasion by residual tumor is overtreatment (Kebebew 2000). In histopathological sections of the first four N0 patients no positive lymph nodes were found and the iCST was negative in these patients. Therefore, we have changed our surgical strategy in patients assessed preoperatively as N0 to a less radical approach and to perform total thyroidectomy with bilateral central node dissection only if iCST is negative. In subsequent patients, ipsilateral dissection of lateral compartments was done only if iCST was positive.

In our study, the iCST was true negative in ten out of eleven MTC N0 and one out of four MTC N+ primarily operated patients. The iCST was true positive in one MTC N0 patient and six out of seven reoperated patients. The test was false negative in two patients preoperativelly evaluated as N+, one primarily operated and one reoperated, respectively. The iCST was true negative in all nine patients with C-cell hyperplasia. Scheuba *et al.* (2007) evaluated the value of iCST in MTC in patients who underwent primary surgery and in patients with no or only central lymph node involvement iCST showed no increase of calcitonin. Similar to our findings, in a group of patients with lymph node involvement in lateral neck, 2 out of 11 patients did not show an increase in calcitonin and were found to have

a single micrometastasis . De Crea *et al.* (2016) in his study in a group of 11 patients showed that iCST can be highly accurate in predicting lateral nodes involvement. Theoretically, both large node-negative and small node-positive tumors can secrete the same quantity of calcitonin (Machens 2008). However, biomarker levels correlate more closely with tumor mass, while lymph node metastases show a markedly weaker correlation with basal calcitonin.

CONCLUSION

In conclusion, the iCST enables an individual aproach to the patient with an early-stage C-cell disease. In patients evaluated in the preoperative period radiologically as N0, a negative result of iCST enables the less radical surgery approach and is a possible marker of good prognosis, however longer follow-up of patients is needed. On the other hand, a positive result for iCST is suggestive of the persistence of tumor tissue and encourages the surgeon to extend the resection and remove residual lymph node micrometastases, while aiming to minimalize the chance of revision neck surgery.

Conflict of interest: *The authors whose names are listed in the title of the article certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, or other equity interest), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.*

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