Sporadic hypercalcitoninemia: clinical and therapeutic consequences

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Abstract

'Calcitonin screening' is not accepted as the standard of care in daily practice. The clinical and surgical consequences of 'calcitonin screening' in a series of patients with mildly elevated basal calcitonin and pentagastrin stimulated calcitonin levels are presented. 260 patients with elevated basal (> 10 pg/ml) and stimulated calcitonin levels (> 100 pg/ml) were enrolled in this prospective study. None of the patients was member of a known medullary thyroid carcinoma family. Thyroidectomy and bilateral central and lateral neck dissections were performed. Testing for the presence of germ-line mutations was performed in all patients. Histological and immunohistochemical findings were compared with basal and stimulated calcitonin levels. All patients were subsequently followed biochemically. C-cell hyperplasia (CCH) was found in 126 (49%) and medullary thyroid cancer was found in 134 (51%) patients. RET proto-oncogen mutations were documented in 22 (8%) patients (medullary thyroid cancer:18, CCH:4). In 56 (46%) of 122 patients, sporadic CCH was classified neoplastic ('carcinoma *in situ*'). Of 97 (72%; 10 with hereditary medullary thyroid cancer) had pT1 (International Union against Cancer recommendations 2002) and 33 (25%) had pT2 or pT3 and 4 (3%) pT4 tumors. Of 39 (29.1%) had lymph node metastases. 106 (79.1%; 15 (38.5%) with lymph node metastases) patients were cured. Evaluation of basal and stimulated calcitonin levels enables the prediction of medullary thyroid cancer. All patients with basal calcitonin > 64 pg/ml and stimulated calcitonin > 560 pg/ml have medullary thyroid cancer. Medullary thyroid cancer was documented in 20% of patients with basal calcitonin > 10 pg/ml but < 64 pg/ml and stimulated calcitonin > 100 pg/ml but < 560 pg/ml.

Endocrine-Related Cancer (2009) 16 243–253

Introduction

Serum calcitonin is the most sensitive biochemical marker for medullary thyroid carcinoma for both primary diagnosis and for follow-up.

Despite the ever increasing number of publications recommending routine calcitonin measurements in patients with thyroid nodules to diagnose medullary thyroid cancer in an early and therefore curable stage

(Pacini *et al.* 1994, Rieu *et al.* 1995, Niccoli *et al.* 1997, Vierhapper *et al.* 1997, 2005, Ozgen *et al.* 1999, Hahm *et al.* 2001, Elisei *et al.* 2004, Papi *et al.* 2006, Costante *et al.* 2007), 'calcitonin screening' is not recommended in daily practice (Hegedus 2004). Differences exist in the literature (Scheuba *et al.* 1999, Iacobone *et al.* 2002, Costante *et al.* 2007) regarding calcitonin cut-off levels, indication, time, and extent of surgery.

This analysis aims to present the clinical and therapeutic implications of routinely performed ‘calcitonin screening’ in a large series of patients with ‘sporadic’ elevated basal and pentagastrin stimulated calcitonin levels.

Patients and methods

Patients

260 consecutive patients (167 males (67%); 93 females (36%): f:m=1: 1.8; Table 1) enrolled in this prospective 10 year study and were diagnosed by a calcitonin

screening program (Vierhapper et al. 1997), demonstrating elevated basal (> 10 pg/ml) and elevated stimulated (> 100 pg/ml) calcitonin levels (Barbot et al. 1994). If basal calcitonin levels were higher than 100 pg/ml, the stimulated calcitonin levels had to be at least twice the basal calcitonin levels (Barbot et al. 1994).

Patients with various nodular thyroid diseases and elevated CT levels were selected for thyroid surgery in four different out-patient departments. No patient was a member of a known medullary thyroid cancer family, or had undergone surgery for medullary thyroid carcinoma.

Table 1 Demographics – medullary thyroid cancer and C-cell hyperplasia

	C-cell hyperplasia (only)			Medullary thyroid cancer		
	Sporadic	Hereditary	Σ	Sporadic	Hereditary	Σ
N	122 (87%)	4 (13%)	126	116 (87%)	18 (13%)	134 (100%)
Female	14 (12%)	1 (25%)	15 (12%)	66	12	78 (58%)
Male	108 (89%)	3 (75%)	111 (88%)	50	6	56 (42%)
Age						
Median ± s.d.	(54.5 ± 12.26)	(60.5 ± 9.74)	(55 ± 12.2)	(59.5 ± 12.7)	(57 ± 20.6)	(59.3 ± 13.9)
Range	(21–75)	(48–69)	(21–75)	(27–83)	(15–79)	(15–83)
Female	14	1	15	66	12	78
Years + s.d.	(45 ± 13.3)	55	(46 ± 12.9)	(59.9 ± 14.2)	(57 ± 20.5)	(59.3 ± 15.2)
Range	(31–75)	55	(31–75)	(27–83)	(24–79)	(24–83)
Male	108	3	111	50	6	56
Years + s.d.	(55.5 ± 12.0)	(66 ± 11.4)	(56 ± 12.0)	(59.3 ± 10.6)	(60.15 ± 22.6)	(59.3 ± 12.3)
Range	(21–74)	(48–69)	(21–74)	(32–81)	(15–73)	(15–81)
pT**						
1a				73 (63%)	8 (44%)	81 (60%)
1b				14 (12%)	2 (11%)	16 (12%)
2				15 (13%)	5 (28%)	20 (15%)
3a				2 (2%)	0	2 (1%)
3b				8 (7%)	3 (17%)	11 (8%)
4a				4 (3%)	0	4 (3%)
4b				0	0	0
Unifocal				93 (80%)	1 (6%)	99 (74%)
Multifocal				23 (20%)	17 (94%)	40 (26%)
pN						
0				86 (74%)	9 (50%)	95 (71%)
1				30 (26%)	9 (50%)	39 (29%)
CCH						
No	0	0	0	52	6	58
Yes	122	4	126	64 (55%)	12 (61%)	76 (56%)
Focal	3	0	3	12	0	12
Diffuse	25	0	25	11	1	12
Nodular	38	1	39	9	5	14
Neoplastic	56	3	59	32	6	38
Cured	122 (100%)	4 (100%)	126 (100%)	95 (82%)	11 (61%)	106 (79%)
if N0				83	8	91
N1				12	3	15
Persisting				21 (18%)	7 (39%)	28 (21%)
disease (M)						
If N0				3	1	4
N1				18	6	24

T, tumor; N, lymph node metastasis; CCH, C-cell hyperplasia; M, distant metastasis; N0, no lymph node metastases; N1, lymph node metastases; M, distant metastases. *UICC 2002/2003.

Biochemical analysis

Calcitonin levels were determined either by an IRMA (IRMA-CIS-Biointernational, Gif-Sur-Yvette, France) or by an immunochemiluminometric assay (ICMA-Nichols Diagnostics, San Juan Capistrano, CA, USA) in one laboratory (Bieglmayer *et al.* 2002).

In the first period of the study, the CIS assay was used and during the second period the Nichols assay was used. Method comparison studies (Bieglmayer *et al.* 2002) demonstrated the CIS and the Nichols assays (correlation coefficient: 0.91) to have the same results (and therefore the same cut-off levels).

Surgery

All patients underwent primary (total) thyroidectomy and microdissection of the central lymph node compartments along both recurrent nerves (central neck dissection; lymph node compartment C1 (Dralle 2002)).

A systematic microdissection of both lateral lymph node compartments (functional lateral neck dissection; compartments C2/C3 (Dralle 2002)) was added if medullary thyroid cancer was established by intraoperative frozen-section.

No transsternal mediastinal dissection (compartment C4 (Dralle 2002)) was performed during initial surgery.

Molecular genetic analysis

The presence of germ-line mutations was tested for all patients by screening exons 8, 10, 11, 13, 14, 15, and 16 of the RET proto-oncogene (Fink *et al.* 1996).

Pathologic examinations

All thyroid glands were submitted to pathology and inspected macroscopically. As recommended by Kaserer *et al.* (2002), the entire organ was sectioned in slices of ~3–5 mm and frozen sections were performed on the macroscopically identified primary tumor. The entire remaining thyroid gland was serially blocked in paraffin. Sections of each block, as well as 3 sections of each submitted lymph node were stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed using the avidin–biotin–peroxidase technique. A section of each block was immunostained for calcitonin, using an available antibody (Chemicon, Temecula, CA, USA) in a dilution of 1:600.

C-cell hyperplasia (CCH) was considered present when at least one area with >50 C-cells per one low power field ($\times 100$) was found in both thyroid lobes. The highest number of C-cells per low power field of each thyroid specimen was recorded. According to the growth pattern of C-cells, CCH was morphologically

classified into focal, diffuse, or nodular (summarized as ‘physiological CCH’; Rosai *et al.* 1992).

Focal CCH was defined by a segmental proliferation of C-cells within thyroid follicles. Diffuse CCH was diagnosed when C-cells formed an intrafollicular, circumferential collar around the more centrally located follicular epithelial cells. Nodular CCH was diagnosed when there was complete obliteration of the follicular lumen by hyperplastic C-cells (Kaserer *et al.* 2002).

‘Neoplastic’ CCH was diagnosed as an additional, separate form of CCH, whenever intrafollicular C-cells with nuclear pleomorphism morphologically distinct from the follicular cells and therefore recognizable on H&E-stained sections were seen.

Areas of C-cell proliferation with suspected early infiltration were regarded as medullary thyroid cancer. C-cell carcinoma was diagnosed, if a focal loss or reduplication of the basement membrane was demonstrated by immunohistochemistry (McDermott *et al.* 1995).

Tumors were staged in accordance with the International Union against Cancer recommendations (UICC) 2002/2003 (Wittekind *et al.* 2002; Wittekind *et al.* 2003).

Postoperative follow-up

All patients were followed clinically and biochemically. Basal and stimulated calcitonin levels were determined at 6 weeks, 6 months and then annually following surgery. Patients appeared to be cured when both the basal and the stimulated calcitonin levels remained below 10 pg/ml 12 months following surgery. Persistently, high basal and/or stimulated calcitonin levels were called ‘persisting’ disease. Elevated basal and/or stimulated calcitonin levels 12 months after previous normalization was defined as ‘recurrent’ disease.

All patients gave informed consent to all diagnostic and therapeutic procedures.

Statistical methods

The SPSS program (Version 12.0.1; 2003 Chicago, IL, USA) was used for statistic analysis. Data is described as the mean $\pm$ s.d. ($\pm$ s.d.). Student’s *t* and χ^2 calculations were applied for comparative analysis. *P* values ≤ 0.05 were considered to be significant.

Results

Morphological results

134 (51.5%) patients had medullary thyroid cancer and 126 (48.5%) patients had CCH (Table 1). More males

($n=111$) than females ($n=15$) had CCH (female:–male=1:7.4; χ^2 Test: $P<0.001$) while more females ($n=78$) than males ($n=56$) had medullary thyroid cancer (female:male=1:0.7; χ^2 Test: $P<0.001$).

Medullary thyroid carcinoma

According to the UICC 2002 (Wittekind *et al.* 2002; Wittekind *et al.* 2003), 81 (60%) of 134 tumors were classified as pT1a (tumor diameter ≤ 10 mm), 16 (12%) as pT1b (11–20 mm), 20 (15%) as pT2 (21–40 mm), 2 (2%) as pT3a (> 41 mm and intrathyroidal), 11 (8%) as pT3b (tumors with minimal extension beyond the thyroid capsule) and 4 (3%) as pT4a (tumor of any size extending beyond the thyroid capsule invading the s.c. soft tissues, larynx, trachea, esophagus, or the recurrent laryngeal nerve (Table 1). No tumor was classified as pT4b (tumor of any size invading the prevertebral fascia or invading the carotid artery or mediastinal vessels)

Mean tumor diameter measured 12.9 ± 4.5 (range: 0.5–90.0) mm.

On average 71 (range 2–188) lymph nodes were dissected, in the medullary thyroid cancer patients. Of 39 patients (29%) had lymph node metastases (Table 1). In those in whom lymph node metastases were found, a mean of 9.4 (range 1–48) lymph nodes were positive.

The frequency and the typing of concomitant CCH are shown in Table 1.

Concomitant CCH was demonstrated immunohistochemically in 76 (56%) patients. 32 (27.6%) patients with sporadic and 6 (33.3%) with hereditary medullary thyroid cancer showed ‘physiological CCH’ while CCH was classified as ‘neoplastic’ in 32 (27.5%) sporadic and 6 (33.3%) hereditary cases respectively.

The pathohistological reports described macroscopic ‘near normal thyroid tissue’ in addition to the foci of sporadic or hereditary medullary thyroid cancer in 40 (29.9%). However, all patients showed micronodules (≤ 2 mm) histologically. One or more hyperplastic macro-nodules (> 2 mm) or diffuse enlargement was described in 74 (55.3%) and 2 (1.4%) glands respectively. Signs of Hashimoto’s thyroiditis were found in 18 (13.4%).

21 (18.1%) of the sporadic and 1 hereditary patient had an associated thyroid carcinoma derived from the follicular epithelium.

By definition, 106 (79%) patients were clinically and biochemically cured by surgery (Table 1). Within the observation period of 4.5 ± 3.06 (0.9–11.7) years, 3 patients died from persistent medullary thyroid cancer 11, 18, and 79 months after surgery respectively.

No clinical or biochemical ‘recurrent’ disease was documented on the follow-up of patients considered to be cured initially.

C-cell hyperplasia

66 (54.1%) sporadic patients showed one of the various ‘physiologic’ and 56 (45.1%) showed ‘neoplastic’ CCH types. All but 1 of the 4 patients with hereditary CCH showed the ‘neoplastic’ variant (Table 1).

In addition to the sporadic or hereditary CCH the pathohistological reports describe macroscopic near ‘normal thyroid tissue’ in 23 (18.2%) patients (definitions see above). One or more hyperplastic macro-nodules were described in 81 (64.3%) patients and diffuse enlargement of the gland in 7 (5.6%) patients respectively. Hashimoto Thyroiditis was diagnosed in 15 (11.9%).

25 (20.5%) sporadic and 2 hereditary patients had an associated thyroid carcinoma derived from the follicular epithelium.

All patients with CCH were biochemically cured.

Molecular genetic results

Table 2 summarizes the molecular genetic findings in 22 patients (8.5%; medullary thyroid cancer: 18 (13.4%) of 134 patients; CCH: 4 (3.2%) of 126 patients) with various RET-proto-oncogen mutations and their genotype–phenotype correlation (Eng 1996).

In 9 patients with hereditary medullary thyroid cancer, a ‘common mutation’ in the extra-cellular domain of the RET-proto-oncogen of exons 10 or 11 was documented, while nine patients had mutations of exons 8, 13, 14, or 15 (‘rare mutations’). All patients with hereditary CCH had mutations of exon 13, codon 791. No patient showed mutations of exon 16.

Correlation of calcitonin levels, C-cell morphology and patients characteristics

In accordance with the ‘receiver operating characteristic curves’ in Scheuba *et al.* (1999), the 260 patients were divided into patients with ‘Mildly’ elevated stimulated calcitonin levels (Group 1 – CCH or medullary thyroid cancer; Biochemical definition/‘cut off’ calcitonin levels: in normal renal function (=creatinine < 1.5 mg/ml); basal calcitonin > 10 pg/ml ≤ 64 pg/ml and stimulated calcitonin > 100 pg/ml < 560 pg/ml) and into patients with ‘Highly’ elevated stimulated calcitonin levels (Group 2 – medullary thyroid cancer 100%; Biochemical definition/cut off calcitonin levels: normal renal function; basal

Table 2 Genotype–phenotype correlation in RET proto-oncogen carrier (index patients)

Exon	Codon	AA exchange		Hereditary medullary thyroid cancer				Hereditary C-cell hyperplasia	
				FMTc	MEN2A			FMTc	Σ
					1	2	3		
8	533	GGC>TGC	gly>cys	1	1			0	1
10	611	TGC>TGG	cys>try	1		1		0	1
	611	TGC>TAC	cys>arg	1		1		0	1
	620	TGC>TAC	cys>arg	1		1		0	1
11	634	TGC>CGC	cys>arg	4	1	1		0	4
	634	TGC>TAC	cys>tyr	2	1			0	2
13	790	TTG>TTT	leu>phe	1	1			0	1
	791	TAT>TTT	tyr>phe	1	1			4	5
14	804	GTG>ATG	val>met	4	3		1	0	4
15	891	TCG>GCG	ser>ala	2	1	1		0	2
Σ				9	3	5	1	4	22
				18				4	

FMTc, medullary thyroid cancer; MEN, multiple endocrine neoplasia; Pheo, pheochromocytoma; PHPT, primary hyperparathyroidism; tyr, tyrosine; phe, phenylalanine; cys, cysteine; trp, tryptophan; leu, leucine; val, valine; ser, serine; met, methionine; MEN2A-1, MTC + Pheo + PHPT; MEN2A-2, MTC + Pheo; MEN2A-3, MTC + PHPT.

calcitonin > 64 pg/ml or basal calcitonin > 10 pg/ml and (independent renal function) stimulated calcitonin ≥ 560 pg/ml; [Table 3](#)).

Group 1: ‘mildly elevated’ stimulated calcitonin levels

Group 1a – medullary thyroid cancer

Medullary thyroid cancer measuring 0.7–7.0 mm was diagnosed by frozen section in 11 (35%) patients. Frozen sections were negative in 20 (65%) patients (diameter: 0.6–7.0 mm) and thus only bilateral central but no lateral functional neck dissections were performed on these patients. All 31 tumors were classified as pT1a. In one (2.9%) female, 4 of 73 lymph nodes showed metastatic involvement.

Group 1b – C-cell hyperplasia

‘Physiological’ CCH was documented in 67 (53%) patients. ‘Neoplastic’ CCH was diagnosed in 59 (47%) patients.

By definition, all patients of group 1a and 1b were biochemically cured.

Group 2: ‘highly’ elevated stimulated calcitonin levels

Medullary thyroid cancer was biochemically predicted preoperatively in all 103 patients.

50 (48.5%) of 103 patients who were classified as pT1a, 38 (36.9%) showed lymph node metastases at the time of diagnosis.

Pentagastrin stimulated calcitonin levels, morphology, gender, and outcome

Increasing stimulated calcitonin levels (steps of 200 pg/ml) demonstrated a highly significant linear trend in the increase of patients with medullary thyroid cancer and a decrease of patients with CCH ($P < 0.0001$; [Table 4](#)).

Overall, more females than males had medullary thyroid cancer ($P < 0.01$; [Table 4](#)). In the two subgroups with stimulated calcitonin levels between 100 and 200 pg/ml and 201–400 pg/ml medullary thyroid cancer was significantly more frequently documented in females than in males ($P < 0.01$; [Table 4](#)): 7 of 18 (39%) and 7 of 9 (78%) females compared with 13 of 90 (14%) and 5 of 36 (14%) males showed medullary thyroid cancer ($P < 0.01$; [Table 4](#)).

Patients with lymph node metastases and with persistent disease corresponding to different stimulated calcitonin subgroups are summarized in [Table 4](#).

Discussion

This is the first single-center series of 260 patients with various nodular thyroid diseases and ‘sporadic hypercalcitoninemia’ that applies the same diagnostic and therapeutic work-up, therefore allowing correlation of biochemical and morphological data and recommendations for therapy. Since the elevated basal and stimulated CT levels were known preoperatively, and therefore MTC was suspected, all patients were treated as if they had MTC.

Table 3 Calcitonin ‘cut off’ levels to predict medullary thyroid cancer^a

PG-test	Calcitonin (pg/ml; cut off levels)			Histology				Σ	
	Basal and stimulated			CCH		MTC			
				<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Group 1 ‘Mildly’ elevated**	> 10	≤64	<560	126	80	31	20	157	60
Group 2 ‘Highly’ elevated**	> 64		Stimulated	0	0	103	100	103	40
	> 10	≤64	≥560	126	48	134	52	260	100

ROC curves see Scheuba *et al.* PG, Pentagastrin stimulation test; CCH, C-cell hyperplasia; MTC, Medullary thyroid cancer.
^aNormal renal function (creatinine ≤ 1.5 mg/ml).

Prevalence findings in the literature – arguments for and against calcitonin screening

A series of original communications from Europe (Pacini *et al.* 1994, Papi *et al.* 2006, Costante *et al.* 2007) followed by editorial comments (Sheppard 1995, Deftos 2004, Elisei *et al.* 2004, Hodak & Burman 2004) discuss the advantages and disadvantages of ‘calcitonin screening’ in patients with (Rieu *et al.* 1995, Ozgen *et al.* 1999, Hahm *et al.* 2001, Iacobone *et al.* 2002, Elisei *et al.* 2004, Vierhapper *et al.* 2005) and without (Karanikas *et al.* 2004) thyroid nodules, in an attempt to diagnose medullary thyroid cancer at an earlier and therefore potentially curable stage (Kebebew *et al.* 2000).

In terms of modern evidence-based medicine requirements, the available level of evidence for ‘calcitonin screening’ and its clinical and therapeutic consequences is limited. ‘Calcitonin screening’ is recommended routinely in patients with thyroid nodular disease in Europe (Sheppard 1995, Niccoli *et al.* 1997, Vierhapper *et al.* 1997, Lips *et al.* 2001) American authors (Deftos 2004, Hodak & Burman 2004) argue against these recommendations. The main arguments against ‘calcitonin screening’ programs include the overall low frequency of medullary thyroid

cancer, screening costs, and the difficulty acquiring pentagastrin for stimulation testing, which is necessary in patients with mild or moderate calcitonin elevation because there is no absolute threshold value for basal calcitonin levels that differentiates benign from malignant C-cell disease (Castro & Gharib 2005).

Routine calcitonin determinations (‘calcitonin screening’) or biopsy, cost effectiveness

Routine calcitonin measurements show a higher sensitivity compared with fine needle aspiration cytology, especially in diagnosing small (≤ 10 mm) medullary thyroid cancer (Pacini *et al.* 1994, Niccoli *et al.* 1997, Ozgen *et al.* 1999, Bugalho *et al.* 2005, Papi *et al.* 2006). The costs of initial ‘calcitonin screening’ in patients with thyroid nodules independent of their size have to be balanced against that of multiple surgical procedures, and arguably worthwhile, but none-the-less frequently used, radiation and chemotherapy in patients with metastatic disease, as well as against the psychological effects of a ‘chronic’ disease (Dunn 1994, Horvit & Gagel 1997, Vierhapper *et al.* 1997). The cost effectiveness of routine serum calcitonin screening of patients undergoing evaluation

Table 4 Correlation of stimulated calcitonin levels, morphology, gender, lymph node involvement and persistence

Stimulated calcitonin (pg/ml)	CCH				MTC			Follow-up persistence n (%)
					Female n (%)		LN pos n (%)	
	n	n (m/f)	n	n	Male n (%)			
100–200	108	90/18	88 (81)	20 (19)	13 (14)*	7 (39)*	3 (15)	1 (5)
201–400	45	36/9	33 (73)	12 (27)	5 (14) [†]	7 (78) [†]	2 (17)	1 (8)
401–600	15	8/7	5 (33)	10 (67)	5 (63)	5 (71)	1 (10)	1 (10)
601–800	6	5/1	0	6 (100)	5 (100)	1 (100)	1 (17)	0
801–	86	28/58	0	86(100)	28(100)	58 (100)	32 (37)	25 (39)
Σ	260	167/93	126 (48)	134 (52)	56 (34)	78 (84)	39 (29)	28 (21)

MTC, medullary thyroid cancer; CCH, C-cell hyperplasia; LN, lymph node; pg, picogramme; ml, milliliter; n, number of patients; m, male; f, female. *Males versus females *P* < 0.01 (χ quadrat test). [†]Males versus females *P* < 0.01 (χ quadrat test).

for thyroid nodules was shown by Shine (2002) and by Cheung *et al.* (2008).

Boi *et al.* (2007) recommend performing calcitonin measurements in wash-out fluids from fine needle aspiration to improve the diagnosis in primary and metastatic medullary thyroid carcinoma in the work-up thyroid nodules to prevent ‘unnecessary’ surgery. However, even this modification with fine needle aspiration evaluation may be inconclusive in ‘calcitonin screened’ patients, because medullary thyroid cancers measured from 0.7 to 7 mm in the group of patients with ‘mildly elevated calcitonin levels’. Medullary thyroid cancer was diagnosed by frozen section in only 11 (35%) of 31 patients.

Normal calcitonin levels and medullary thyroid cancer

Normal basal calcitonin values of <10 pg/ml practically exclude medullary thyroid cancer (Pacini *et al.* 1994, Rieu *et al.* 1995, Niccoli *et al.* 1997, Kaserer *et al.* 1998, Ozgen *et al.* 1999, Hahm *et al.* 2001, Iacobone *et al.* 2002). Therefore, basal calcitonin levels of ≥ 10 pg/ml were used as the initial diagnostic tool to stratify for medullary thyroid cancer risk and this was defined as upper normal reference limit in most of the studies (Demers & Spencer 2003). Elevation in the basal calcitonin levels alone is not evidence of medullary thyroid cancer (Costante *et al.* 2007). Stimulation tests (Demers & Spencer 2003) are mandatory to confirm this suspicion. Patients with stimulated calcitonin levels of 30–100 pg/ml have a low risk (<3%) for sporadic medullary thyroid cancer (Barbot *et al.* 1994, Iacobone *et al.* 2002). The risk for medullary thyroid cancer is greater than 50% in patients with stimulated calcitonin values >100 pg/ml (Costante *et al.* 2007), quoting a minimal prevalence of medullary thyroid cancer of 0.26–1.37% (Pacini *et al.* 1994, Rieu *et al.* 1995, Niccoli *et al.* 1997, Vierhapper *et al.* 1997, Ozgen *et al.* 1999, Hahm *et al.* 2001, Papi *et al.* 2006, Costante *et al.* 2007). A higher stimulated calcitonin cut-off level (e.g., 200 pg/ml (Iacobone *et al.* 2002) would further increase the positive predictive value for medullary thyroid cancer, but more than a third of medullary thyroid cancers would escape diagnosis and treatment (Iacobone *et al.* 2002). Thus, the definition of 100 pg/ml as the therapeutic threshold reflects a clinical compromise, balancing over- and under-treatment in these patients. A strict interpretation of basal with stimulated calcitonin levels further increases the positive predictive value for the presence of medullary thyroid cancer (Scheuba *et al.* 1999).

Elevated calcitonin levels and C-cell hyperplasia

Elevated stimulated calcitonin levels (>100 pg/ml) were either associated with medullary thyroid cancer (51.2%) or with various types of CCH (48.8%). There were no false-positive tests regarding these two entities when applying defined cut-off levels and strictly individualized combined interpretation of basal and stimulated calcitonin levels (Scheuba *et al.* 1999).

Age and sex both influence plasma calcitonin levels in normal human subjects (Deftos *et al.* 1980, Hahm *et al.* 2001). Calcitonin was generally higher in men than in women in each age group (Deftos *et al.* 1980, Hahm *et al.* 2001). Irrespective of age, the increase in calcitonin in response to stimulation was greater in men than in women (Deftos *et al.* 1980). Basal calcitonin levels and the response to stimulation waned with age in both sexes (Deftos *et al.* 1980).

Various types of CCH may be documented concomitantly to different benign and malignant thyroid disorders (Scheuba *et al.* 2000). No age- or gender-related differences in the occurrence of CCH in normal basal calcitonin and stimulated calcitonin levels were observed (Scheuba *et al.* 2000).

The term ‘CCH’ refers to the (reactive) ‘physiological’ and ‘neoplastic’ variants. By definition, 67 (54.9%) of the 122 patients with verified CCH morphologically fulfilled the criteria of ‘physiological’ and 59 (48.3%) of ‘neoplastic’ CCH.

In 1 of 4 patients with RET proto-oncogen mutations, CCH was classified as ‘physiologic’, 3 as ‘neoplastic’. Besides sporadic medullary thyroid cancer, ‘physiological’ and ‘neoplastic’ CCH was diagnosed in 32 (27.6%) patients each, while histological examinations revealed ‘physiological’ CCH in six and ‘neoplastic’ CCH in another 6 of 18 patients with hereditary medullary thyroid cancer. In six patients no CCH was documented.

Different neoplastic potential is discussed for these two different pathologic conditions (Perry *et al.* 1996, LiVolsi 1997, Albores-Saavedra & Krueger 2001). ‘Neoplastic’ CCH (so-called C-cell carcinoma *in situ*) is generally accepted as the precursor lesion of hereditary MTC, associated with germ-line mutations in the RET proto-oncogene. However, in our study, ‘neoplastic’ CCH was not documented in all patients with hereditary CCH or hereditary medullary thyroid cancer, but also in patients without genetic alterations.

The malignant potential of (reactive) ‘secondary’ or ‘physiologic’ CCH has not been fully demonstrated and its clinical relevance in the development of sporadic medullary thyroid cancer remains unclear (LiVolsi 1997, Verga *et al.* 2007). However, in one

patient, a state of incipient medullary thyroid cancer emerging from nodular CCH was documented (Vierhapper et al. 1997). The hypothetical role of CCH as a risk factor for sporadic C-cell malignancy (Kaserer et al. 1998) is not supported by available evidence (LiVolsi 1997, Kaserer et al. 2002). Therefore, the clinical utility of histopathological documentation of the different variants of CCH particularly the ‘neoplastic’ variant to indicate genetic medullary thyroid cancer risk, is small as up to 50% of patients with sporadic medullary thyroid cancer or with sporadic CCH may have by definition (LiVolsi 1997) the ‘neoplastic’ CCH.

Elevated calcitonin levels and medullary thyroid cancer

In this study, various types of CCH were more common in males than in females including patients with bCT levels >10 pg/ml and sCT levels >100 pg/ml. The distinction of ‘neoplastic CCH’ from microinvasive MTC may be very difficult in individual cases. Preoperatively, they cannot be discriminated biochemically (Scheuba et al. 1999, 2007). Morphologically ‘neoplastic’ CCH is characterized by groups of intrafollicular atypical CC with partial or complete obliteration of the follicular spaces (LiVolsi 1997). There is a progressive increase of the proliferation index accompanied by an increase of molecular alterations and monoclonality, leading to invasive MTC (Matias-Guiu et al. 1995). The presence of fibrosis around tumor cell nests and demonstration of defects in the follicular basement membrane by immunohistochemistry or electron microscopy may be helpful to definitively discriminate both entities (McDermott et al. 1995).

Medullary thyroid cancer was documented in 134 (51.2%) of 260 patients. 103 (76.9%) medullary thyroid tumors could be predicted biochemically during preoperative evaluation.

Without routine calcitonin measurements, medullary thyroid cancer will be identified as a palpable thyroid nodule reflecting a more advanced disease. The presence of lymph node and distant metastases at first diagnosis significantly worsens the prognosis (Kebebew et al. 2000, Scollo et al. 2003). Metastases to lymph nodes in the neck appear early. 39 (29.1%) of 134 patients had lymph node metastases. Their frequency is roughly proportional to the size of the primary tumor. Hence, 10 (12.3%) of 81 pT1 tumors had lymph node metastases at initial diagnosis. Although, patients with basal calcitonin <22 pg/ml were all node negative (Scheuba et al. 1999), neither

medullary thyroid cancer nor lymph node metastases could be predicted biochemically (Scheuba et al. 1999). Therefore, thyroidectomy and bilateral central neck dissection should be performed as the minimal initial treatment in patients selected for surgery by ‘calcitonin screening’.

By definition, 106 (79%) patients were clinically and biochemically cured by surgery. 15 (38.5%) of 39 patients with lymph node metastases showed undetectable basal and stimulated calcitonin levels.

Clinical and therapeutic consequences and recommendations

Surprisingly, a better knowledge of the unique biochemical, molecular, genetic, and clinical features of medullary thyroid cancer neither lead to earlier diagnosis and treatment nor to improvement of survival in the USA (Kebebew et al. 2005). A high proportion of patients still receive less than optimal initial surgical treatment (Kebebew et al. 2005), especially when medullary thyroid cancer is small and therefore clinically unexpected.

‘Calcitonin screening’ in patients with solitary and multiple thyroid nodules improves the early detection of (clinically unexpected) medullary thyroid cancer. This may be useful in assessing for tumor spread preoperatively and may help in evaluation, allowing for a stage adapted, and therefore potentially curable, surgical procedure.

Medullary thyroid cancer can be predicted with 100% certainty in patients with ‘highly’ elevated stimulated calcitonin levels. Therefore, thyroidectomy with bilateral central (C1) and bilateral (functional) lateral neck dissection (C1–C3) is recommended as lymph node positive and lymph node negative patients cannot be differentiated biochemically. Bilateral, central, and lateral lymph nodes may be affected independent of the size, number, and location of the tumor and micrometastases may not be detectable by ultrasound. Involvement of mediastinal lymph nodes (C4) may be present. Transsternal mediastinal dissection may be performed in patients with a positive postoperative pentagastrin test during a second operation following the exclusion of distant metastases.

Differences exist in the proposals that address, cut-off levels, time, and extent of surgery in patients with ‘mildly’ elevated basal and stimulated calcitonin levels (Scheuba et al. 1999, Iacobone et al. 2002). Currently, biochemical differentiation between patients with ‘C-cell hyperplasia only’ and those with medullary thyroid microcarcinoma cannot be made preoperatively.

Medullary thyroid cancer may sometimes not be diagnosed by frozen section in patients with ‘mildly’ elevated stimulated calcitonin levels, and therefore thyroidectomy and bilateral central neck dissection (C1) is the treatment of choice. Medullary thyroid carcinoma exists in only 20% in this ‘gray zone’ where CCH and medullary thyroid cancer overlap.

In contradistinction with Iacobone (Iacobone *et al.* 2002) who proposed C1-dissection only in patients with stimulated calcitonin levels >200 pg/ml, a central neck dissection is recommended in all patients with stimulated calcitonin levels >100 pg/ml because lymph node metastases were documented in 3 (15%) of 20 patients with stimulated calcitonin levels between 100 and 200 pg/ml. A bilateral lateral neck dissection (C1–C3) may be performed ‘on demand’ after thyroidectomy and negative or positive C1-dissection, if postoperative basal calcitonin and/or stimulated calcitonin levels are measurable, indicating lymph node metastases outside the central neck.

Conclusions

During work-up of thyroid nodules routinely, the evaluation of basal and stimulated calcitonin levels additionally performed in patients with elevated basal hormone levels (‘calcitonin screening’) enables the prediction of medullary thyroid cancer. By definition, all patients (100%) with ‘highly elevated’ calcitonin levels (basal calcitonin >64 pg/ml; stimulated calcitonin >560 pg/ml) suffer from medullary thyroid cancer. Therefore, ‘adequate’ curative primary surgery can be offered to these patients. Medullary thyroid cancer was documented in at least 20% of patients with ‘mildly elevated’ calcitonin levels (basal calcitonin >10 pg/ml but <64 pg/ml and stimulated calcitonin >100 pg/ml but <560 pg/ml). In this select group, females are significantly more commonly affected by medullary thyroid cancer than males are. Therefore, a more ‘liberal indication’ for surgery is recommended in females with stimulated calcitonin levels up to 400 pg/ml.

Declaration of interest

I declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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