Calcitonin concentrations in patients with chronic kidney disease and medullary thyroid carcinoma or c-cell hyperplasia

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It is currently not known which level of pentagastrin-stimulated calcitonin serum concentration indicates medullary thyroid carcinoma in patients with chronic kidney disease (CKD). We examined CKD stage 3–5 patients who had total thyroidectomy because of a pentagastrin-stimulated calcitonin concentration greater than 100 pg/ml, and tested the diagnostic performance of basal and pentagastrin-stimulated calcitonin levels for differentiating medullary thyroid carcinoma and C-cell hyperplasia in this patient population. A total of 180 CKD patients presented with an elevated calcitonin level and had a pentagastrin stimulation test. Forty patients showed a maximum pentagastrin-stimulated calcitonin concentration greater than 100 pg/ml, and 22 patients had a total thyroidectomy. Seven of these 22 patients presented with a medullary thyroid carcinoma, all other patients showed C-cell hyperplasia. Patients with medullary thyroid carcinoma showed higher unstimulated (212 pg/ml (36–577) vs 42 pg/ml (17–150); $P<0.001$) and higher maximum pentagastrin-stimulated calcitonin concentrations (862 pg/ml (431–2423) vs 141 pg/ml (102–471); $P<0.001$) as compared to patients with C-cell hyperplasia. The sensitivity (100%) and specificity (93%) estimates suggested that a maximum pentagastrin-stimulated calcitonin concentration greater than 400 pg/ml indicates the presence of medullary thyroid carcinoma in patients with CKD. Receiver-operating characteristic (ROC) analysis revealed an area under the ROC plot of 0.99 for maximum pentagastrin-stimulated calcitonin concentrations. A maximum pentagastrin-stimulated calcitonin concentration greater than 400 pg/ml appears to be a clinically meaningful threshold for thyroidectomy.

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The C cells of the thyroid gland produce calcitonin in response to hypercalcemia, thus contributing to calcium homeostasis.[1] A calcitonin level greater than 10 pg/ml and an increase of calcitonin in response to stimulation with pentagastrin above 100 pg/ml (positive pentagastrin test) may indicate the presence of medullary thyroid carcinoma. Total thyroidectomy is recommended in such cases.[2] Benign C-cell hyperplasia, however, may also be associated with elevated calcitonin concentrations.

Among patients with chronic kidney disease (CKD), the calcitonin concentration is elevated in about 30%.[3–6] It is currently not known which level of pentagastrin-stimulated calcitonin indicates medullary thyroid carcinoma, or reflects only C-cell hyperplasia and secondary hypercalcitoninemia in CKD. Recently, we demonstrated that pentagastrin-stimulated calcitonin levels of around 100 pg/ml normalize after successful kidney transplantation.[7] We then hypothesized that pentagastrin-stimulated calcitonin concentrations far above 100 pg/ml may not necessarily reflect the presence of medullary thyroid carcinoma in CKD patients and pointed to the need for further studies that examine calcitonin levels in CKD patients with histologically proven medullary thyroid carcinoma or C-cell hyperplasia.

We examined CKD patients of our institution who underwent total thyroidectomy and tested the diagnostic performance of basal and pentagastrin-stimulated calcitonin levels for differentiating medullary thyroid carcinoma and C-cell hyperplasia.

RESULTS

Patients

In the years from 1997 to 2004, 180 CKD patients who presented with an elevated calcitonin level had a pentagastrin stimulation test. Forty patients showed a maximum pentagastrin-stimulated calcitonin concentration greater than

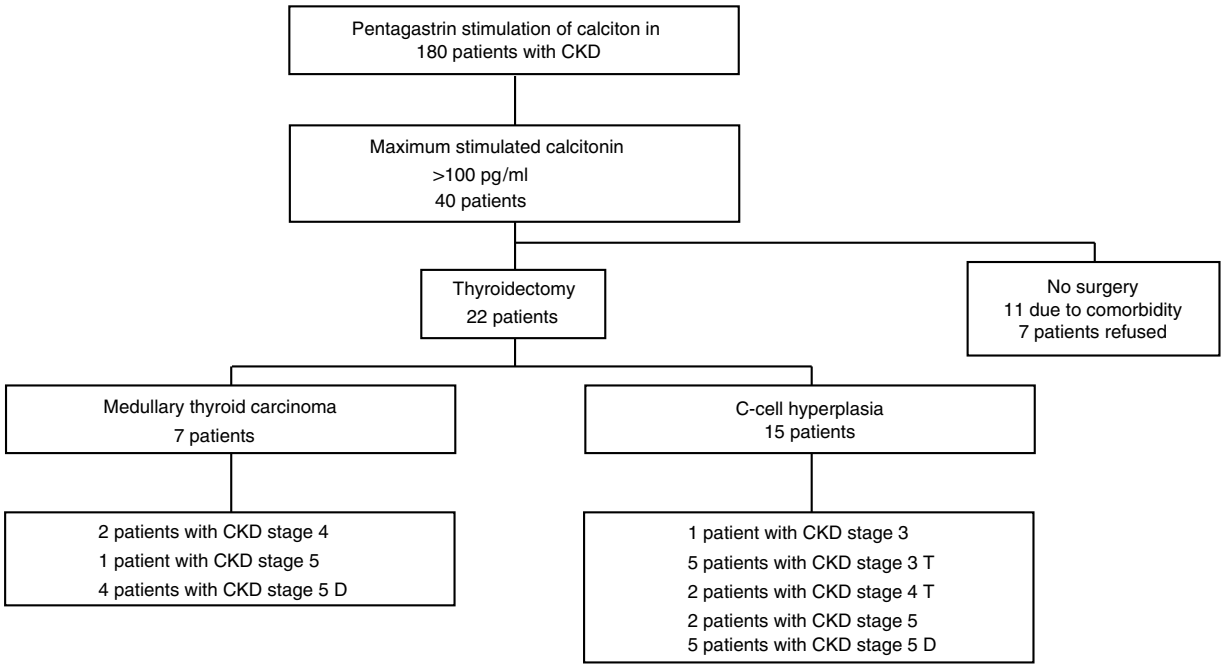

Figure 1 | Flow of patients with CKD and elevated calcitonin levels. T, transplantation, D, dialysis.

100 pg/ml, and 22 patients had a total thyroidectomy (for details of patient flow, see Figure 1).

Important demographic data and characteristics of the 22 patients with thyroidectomy are indicated in Table 1. At the time of calcitonin screening, nine patients were on dialysis (for 1.4 years (0.1–7.8), seven had a kidney graft (first renal replacement therapy was 7.3 years (2.4–10.5) ago), and six patients not on renal replacement therapy had CKD stage 3–5. Thyroid nodules or diffuse enlargement were palpable in six patients, and detected by ultrasonography in further eight patients. Calcitonin concentrations at screening and the basal and maximum pentagastrin-stimulated calcitonin concentrations of the entire cohort are indicated in Table 2.

Table 1 | Demographic and clinical characteristics of 22 patients with CKD at the time point of calcitonin screening who later had a pentagastrin-stimulated calcitonin greater than 100 pg/ml and total thyroidectomy

	Median (min–max)/ Count (frequency)
Age (years)	53 (18–72)
Gender (male)	21
Current renal function/RRT modality	
Hemodialysis	7 (32)
Peritoneal dialysis	2 (9)
Transplantation (eGFR: 44 ml/min/1.73 m^2; 20–59)	7 (32)
CKD (eGFR: 18 ml/min/1.73 m^2; 12–45)	6 (27)
Native kidney disease	
Glomerulonephritis	4 (18)
Diabetic nephropathy	3 (14)
Polycystic kidney disease	2 (9)
Others	9 (41)
Unknown	4 (18)
Calcitonin (pg/ml)	60 (17–577)
Parathyroid hormone (pg/ml)	180 (45–1300)
Calcium (mmol/l)	2.3 (2.10–2.69)
Phosphorus (mmol/l)	1.6 (0.81–2.82)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy.

Surgery

Total thyroidectomy with central and bilateral dissection of cervical lymph nodes was performed in all patients 3.3 (0.2–40) months after the positive pentagastrin stimulation test. In seven patients, thyroidectomy was combined with total removal of the parathyroid glands and autotransplantation of one gland in the forearm, in 10 patients with subtotal removal of the parathyroid glands, and five patients had no surgery of the parathyroid glands.

Histological findings

Seven of the 22 patients undergoing thyroidectomy presented with a medullary thyroid carcinoma (Figure 2a), all other patients showed C-cell hyperplasia (Figure 2b and c). Characteristics of all patients with medullary thyroid carcinoma are indicated in Table 3. Lymph node metastases of medullary thyroid carcinoma were found in one patient (two out of 72 examined lymph nodes). There was no sign of distant metastases in any of the patients. No medullary thyroid carcinoma was found in patients after kidney transplantation. None of the patients with medullary thyroid carcinoma had germline mutations of the ret protooncogene.

Table 2 | Comparison of CKD patients with medullary thyroid carcinoma or with C-cell hyperplasia at the time of calcitonin screening, and results of pentagastrin stimulation

	Median (min–max)/Count	
	MTC (*n*=7)	CCH (*n*=15)
Age (years)	56 (48–72)	53 (18-65)
Gender (male)	6	15
CT at screening (pg/ml)	212 (36–577)	42 (17–150)*
CT at baseline (pg/ml)	311 (40–545)	35 (22–163)**
CT maximum stimulated (pg/ml)	862 (431–2423)	141 (102–471)***

CCH, C-cell hyperplasia; CKD, chronic kidney disease; CT, calcitonin; MTC, medullary thyroid carcinoma.
*$P<0.001$, **$P<0.001$, *** $P<0.001$.

Seven out of 22 patients had incidental papillary carcinomas of the thyroid glands (three of them accompanied medullary thyroid carcinomas). Lymph node metastases of papillary thyroid carcinoma were found in five patients (no distant metastases). For further details, see Table 3.

Calcitonin concentrations in patients with medullary thyroid carcinoma or C-cell hyperplasia

Pentagastrin-stimulated calcitonin concentrations of patients with medullary thyroid carcinoma and of patients with C-cell hyperplasia are shown in Figure 3a and b. Patients presenting with medullary thyroid carcinoma showed higher unstimulated ($P<0.001$) and higher maximum pentagastrin-stimulated calcitonin concentrations ($P<0.001$) as compared to patients with C-cell hyperplasia (Table 2).

Receiver-operating characteristic analysis

Receiver-operating characteristic (ROC) analysis revealed an area under the ROC plot of 0.93 for unstimulated and of 0.99 for maximum pentagastrin-stimulated calcitonin concentrations. The ROC curves are shown in Figure 4. Sensitivities and specificities for different diagnostic thresholds of unstimulated and pentagastrin-stimulated calcitonin concentrations are indicated in Table 4 and 5.

Follow-up of patients without thyroidectomy

Eighteen patients with a positive pentagastrin stimulation test were not referred to surgery. Seven of these patients refused surgery and 11 had comorbid conditions precluding thyroid surgery. Meanwhile, five patients died, all because of cardiovascular comorbidity (aged 57–93 years), two with stable and three without follow-up of unstimulated calcitonin concentrations.

Thirteen patients had repeated pentagastrin stimulation tests with basically same results. Maximum stimulated calcitonin concentration at study time point was 168 ± 77 and 153 ± 72 pg/ml at follow-up investigation 1.1 years later (for further details, see Table 6). In none of the patients, a clinical sign of medullary thyroid carcinoma was apparent. One patient (Table 6, ID 7) who primarily refused surgery had thyroidectomy 1.2 years after the first pentagastrin stimulation test. The histology revealed a neoplastic C-cell hyperplasia.

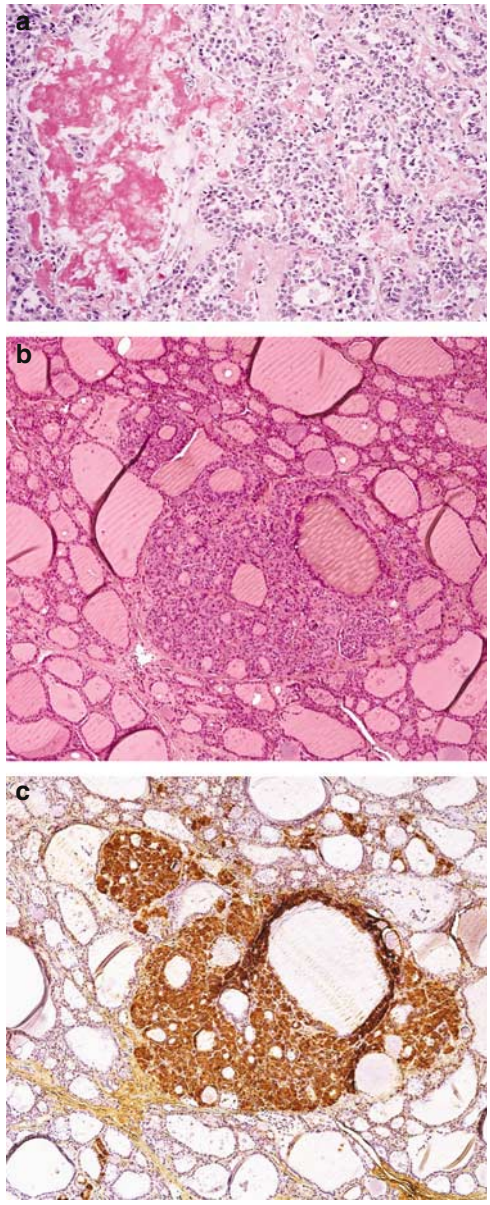

Figure 2 | Thyroid pathology. (**a**) High power field showing solid nests and columns of typical small round cells of a medullary thyroid carcinoma (hematoxylin and eosin (H&E), original magnification $\times 100$). (**b**) Low power view showing nodular C-cell hyperplasia surrounded by unchanged follicles and colloid of the thyroid gland (H&E, original magnification $\times 40$). (**c**) Same view of C-cell hyperplasia with calcitonin staining (immunostain, original magnification $\times 40$).

DISCUSSION

We provide evidence that patients with CKD and medullary thyroid carcinoma have higher unstimulated and higher pentagastrin-stimulated serum calcitonin concentrations than patients with CKD and C-cell hyperplasia. A maximum pentagastrin-stimulated calcitonin concentration exceeding 400 pg/ml in patients with CKD indicates the presence of medullary thyroid carcinoma, whereas in patients with CKD and a maximum pentagastrin-stimulated calcitonin

Table 3 | Pathology of the thyroid gland in 22 chronic kidney disease patients

Patient number	Age (years)	Sex	CKD stage	Palpable thyroid abnormality	Nodule size by sonography (Maximum diameter (mm))	C cells (Count (per low-power field))	Thyroid pathology	
							Medullary C-cell pathology (tumor stages or type of CCH)	Incidental papillary tumors (tumor stages)
1	18	M	5	Soft nodules	29	158	Diffuse	pT1b pN1b M0
2	22	M	5D	Normal	—	111	Diffuse	—
3	31	M	4T	Normal	6	764	Diffuse and nodular	—
4	42	M	5D	Normal	ND	386	Neoplastic	—
5	42	M	5D	Normal	6	305	Diffuse and nodular-neoplastic	pT2a pN1b M0
6	49	M	3T	Normal	—	506	Nodular-neoplastic	—
7	52	M	4T	Small nodules	11	264	Diffuse	—
8	53	M	3	Normal	—	851	Neoplastic	pT4a pN1b M0
9	54	M	3T	Enlarged, cystic	56	465	Diffuse and nodular	—
10	55	M	5	Normal	6	658	Neoplastic	pT1a N0 M0
11	59	M	3T	Normal	10	269	Neoplastic	—
12	59	M	5D	Normal	5	354	Nodular	—
13	61	M	5D	Small nodules	20	671	Neoplastic	—
14	64	M	3T	Normal	5	645	Diffuse, nodular-neoplastic	—
15	65	M	3T	Normal	ND	786	Nodular-neoplastic	—
16	48	M	5D	Normal	ND	807	*pT1b pN0 M0	—
17	52	M	5D	Normal	7	89	*pT1a pN0 M0	pT1b pN1a M0
18	53	M	5	Normal	—	957	*pT1a pN0 M0	—
19	56	M	5D	Normal	3	477	*pT1a pN0 M0	PT1a pN1b M0
20	60	M	4T	Normal	—	ND	pT1b pN0 M0	—
21	70	M	5D	Enlarged, cystic	42	63	*pT1a pN1a M0	—
22	72	F	4	Multiple nodules	15	25	pT2 pN0 M0	pT1b pN0 M0

CCH, C-cell hyperplasia; CKD, chronic kidney disease; D, dialysis; F, female; M, male; ND, not done; T, transplantation.
*Patients presenting with medullary mircrocarcinoma, that is, maximum diameter of tumor <10 mm.

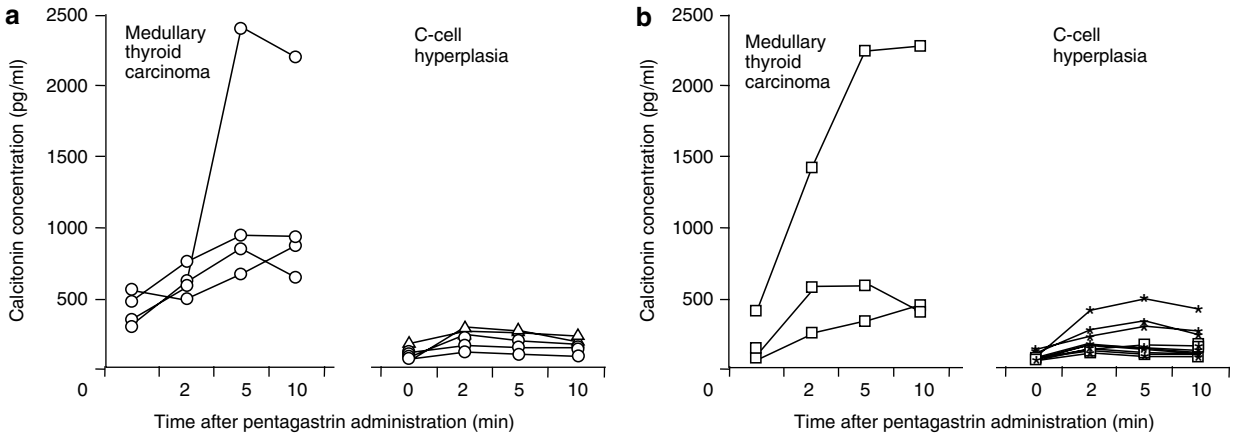

Figure 3 | Basal and pentagastrin-stimulated serum calcitonin concentrations of 22 patients with CKD presenting with either C-cell hyperplasia or medullary thyroid carcinoma. (**a**) Patients with CKD stage 5 D either on hemodialysis (○) or peritoneal dialysis (△). T, transplantation, D, dialysis. (**b**) Patients with CKD stages 3–5 (□) or CKD stage 3–4 T (*).

concentration of less than 400 pg/ml, the presence of C-cell hyperplasia is likely.

Medullary thyroid carcinomas derive from the calcitonin-secreting thyroid C cell. Twenty percent occur as inherited syndromes, including multiple endocrine neoplasia type 2A, multiple endocrine neoplasia type 2B, and familial non-multiple endocrine neoplasia medullary thyroid carcinoma,[8] whereas the majority occurs as sporadic disease (80%). C-cell pathology, including physiologic/reactive C-cell hyperplasia, neoplastic C-cell hyperplasia, and medullary microcarcinoma, can be detected biochemically by measuring calcitonin serum concentrations, both with and without pentagastrin

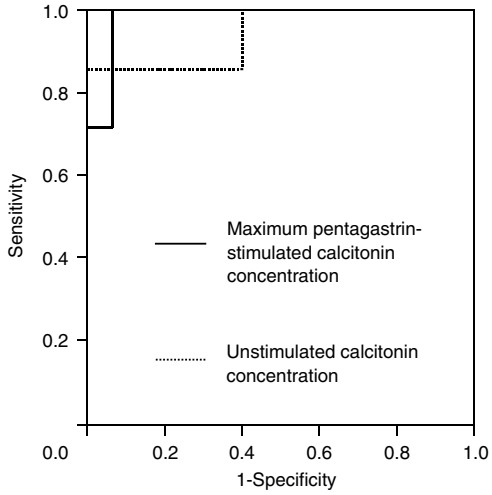

Figure 4 | ROC curves of unstimulated and maximum pentagastrin-stimulated calcitonin concentrations.

Table 4 | Sensitivity and specificity estimates of different basal calcitonin threshold levels for presence of medullary thyroid carcinoma.

Basal calcitonin (pg/ml)	Sensitivity (95% CI)	Specificity (95% CI)
10	100 (59–100)	0 (0–22)
30	100 (59–100)	27 (8–55)
40	100 (59–100)	60 (32–84)
50	86 (42–100)	67 (38–88)
100	86 (42–100)	87 (60–98)
150	71 (29–96)	93 (68–100)
200	71 (29–96)	100 (78–100)
300	57 (18–90)	100 (78–100)
400	29 (4–71)	100 (78–100)
500	14 (0–58)	100 (78–100)

Table 5 | Sensitivity and specificity estimates of different maximum pentagastrin-stimulated calcitonin threshold levels for presence of medullary thyroid carcinoma

Maximum calcitonin (pg/ml)	Sensitivity (95% CI)	Specificity (95% CI)
100	100 (59–100)	0 (0–22)
200	100 (59–100)	60 (32–84)
300	100 (59–100)	87 (60–98)
400	100 (59–100)	93 (68–100)
500	86 (42–100)	100 (78–100)
800	71 (29–96)	100 (78–100)

and/or calcium stimulation. Calcitonin testing, together with mutational analysis of RET, is a well-established tool for the management of families with hereditary forms of medullary thyroid carcinoma or for follow-up after surgery for sporadic medullary thyroid carcinoma.[9–11] In contrast, the role of routine measurement of calcitonin in patients with thyroid nodules, or screening of other patient populations is a matter of debate. Although recommended by several experts, the cost-effectiveness of calcitonin screening is not proven and the results can be misleading.[11–14] Nevertheless, surgical treatment was recommended if calcitonin levels surpass 100 pg/ml.[2,15]

The situation of calcitonin testing in patients with CKD is even more puzzling. Up to 30% of patients with advanced renal insufficiency present with elevated calcitonin concentrations[3–6] and calcitonin stimulation with pentagastrin or calcium revealed controversial results in such patients.[3–6,16] A recent study examined kidney transplant wait-listed patients with CKD stage 4 and stage 5, who, in several cases, presented with elevated pentagastrin-stimulated calcitonin levels close to 100 pg/ml (a suggested threshold for thyroid surgery). After successful kidney transplantation, unstimulated and pentagastrin-stimulated calcitonin concentrations returned to normal in almost all patients.[7] Thus, it was concluded that calcitonin elevation not necessarily reflects accumulation of calcitonin owing to decreased renal clearance, but may be due to increased calcitonin secretion related to C-cell hyperplasia in uremia (secondary hypercalcitoninemia). We mentioned that calcitonin levels close to 100 pg/ml obviously do not indicate presence of medullary thyroid carcinoma in patients with advanced kidney disease. We also pointed to the need for studies correlating elevated stimulated and unstimulated calcitonin levels with histologically proven C-cell hyperplasia and medullary thyroid carcinoma in such patients.[7]

In the present study, we examined the diagnostic accuracy of unstimulated and pentagastrin-stimulated calcitonin levels for differentiating medullary thyroid carcinoma and C-cell hyperplasia in patients with CKD and advanced renal failure (estimated glomerular filtration rate <60 ml/min/1.73 m^2 or dialysis), who had a total thyroidectomy because of a positive pentagastrin stimulation test. The histological examination of total thyroidectomy specimens of 22 patients showed seven cases with medullary thyroid carcinoma. Patients of our study who had histologically proven medullary thyroid carcinoma had higher calcitonin concentrations than patients presenting with C-cell hyperplasia.

ROC analysis of basal and maximum stimulated calcitonin concentration suggested a diagnostic accuracy, that is, the ability to discriminate between medullary thyroid carcinoma and C-cell hyperplasia of 50–150 pg/ml for basal calcitonin concentration and 400–500 pg/ml for maximum stimulated calcitonin concentration. The area under the ROC plot was excellent in both instances. In patients on dialysis, however, unstimulated calcitonin concentrations seem to be higher than in patients with CKD without need for dialysis. As one can see from Figure 3a, medullary thyroid carcinoma was found in patients on dialysis only when unstimulated calcitonin concentration exceeded 250 pg/ml. Presuming the patient's general condition is suitable for surgery, it seems reasonable to perform a thyroidectomy in patients with a pentagastrin-stimulated calcitonin concentrations greater than 400 pg/ml. Follow-up of patients with positive

Table 6 | Follow-up on pentagastrin stimulation in 13 patients with CKD who denied recommended surgery or who were not eligible for surgery due to comorbidity

ID	Age (years)	First calcitonin stimulation (pg/ml)					Second calcitonin stimulation (pg/ml)					Time between both tests (years)
		0	2 min	5 min	10 min	Max	0	2 min	5 min	10 min	Max	
1	77	23	138	113	85	138	22	57	58	47	58	0.
2	52	22	90	101	85	101	28	114	113	113	114	0.8
3	47	30	102	88	75	102	19	84	81	56	84	1.1
4	59	41	98	90	62	98	38	116	116	78	116	0.2
5	61	50	52	106	87	106	23	107	90	66	107	2.5
6	47	55	229	225	138	229	57	263	269	251	269	0.7
7	54	26	128	112	79	128	32	124	110	82	124	1.2
8	70	38	151	199	14	199	61	262	227	183	262	0.5
9	58	40	190	156	114	190	41	165	130	109	165	0.6
10	63	50	98	309	282	309	44	87	218	206	218	0.5
11	44	66	174	150	116	174	65	153	124	116	153	0.6
12	38	17	100	91	54	100	16	80	65	41	80	4.1
13	24	58	315	265	192	315	58	295	240	196	240	1.1

CKD, chronic kidney disease.

pentagastrin stimulation test not undergoing thyroidectomy showed stable calcitonin concentrations. This finding further supports the presence of secondary hypercalcitoninemia in patients with CKD.

In three patients with medullary thyroid carcinoma and in four patients with C-cell hyperplasia, we found an incidental papillary thyroid microcarcinoma in the surgical specimens. The relationship between incidental papillary microcarcinoma and clinical papillary thyroid carcinoma is not clear, but both entities may differ in terms of etiology and biologic behavior. Autopsy studies showed that occult papillary thyroid carcinoma is present in 1–36% of patients who had no known history for thyroid disease.[17] Therefore, a more conservative approach to manage incidental papillary carcinoma was suggested to be wise.[17] In patients without CKD, co-occurrence of medullary thyroid carcinoma with papillary microcarcinoma was reported recently.[18] However, in a large series from Italy the presence of papillary microcarcinoma did not alter the clinical course as compared to the occurrence of medullary thyroid carcinoma alone.[18]

The small sample size of patients who underwent thyroid surgery is a limitation to our study. Nevertheless, this analysis represents the largest case series of CKD patients presenting with medullary thyroid carcinoma. A further limitation to our study might be the under-representation of women. There exists the possibility of overseeing women with C-cell hyperplasia and/or medullary thyroid carcinoma when an unstimulated calcitonin concentration threshold of 10 pg/ml is used as an indication for pentagastrin stimulation instead of the upper limit of the reference interval.

This study of patients with CKD for the first time provides an analysis of diagnostic accuracy of basal and pentagastrin-stimulated calcitonin levels for medullary thyroid carcinoma in patients with CKD. Our data suggest that a maximum pentagastrin-stimulated calcitonin concentration greater than 400 pg/ml indicates the presence of medullary thyroid carcinoma in patients with CKD.

MATERIALS AND METHODS

Study design

In patients with CKD, serum calcitonin concentrations are determined as standard of care at our institution to exclude the presence of medullary thyroid carcinoma. At calcitonin concentrations above 10 pg/ml (close or greater than 30 pg/ml in dialysis patients), a pentagastrin stimulation test is recommended. If the maximum increase of serum calcitonin after pentagastrin stimulation exceeds 100 pg/ml, total thyroidectomy has been performed in the past. We included in this analysis all CKD patients of our institution who had a positive pentagastrin stimulation test and subsequent thyroidectomy and compared basal and pentagastrin-stimulated calcitonin levels in patients presenting with either medullary thyroid carcinoma or with C-cell hyperplasia.

Calcitonin screening

Routine calcitonin screening was done together with parathyroid hormone measurements in patients on hemodialysis every 3 months. In patients on peritoneal dialysis, it was done once a year. In CKD patients stage 3–5, the decision was made by the individual physician in charge. In case the patient was listed for a kidney transplantation or had a kidney transplantation, calcitonin was measured once a year.

For the pentagastrin stimulation test, fasting blood was drawn before and 2, 5, and 10 min after an intravenous administration of a bolus of 0.5 μg/kg body weight pentagastrin (Peptavlon, Astra-Zeneca, Vienna, Austria) for the determination of serum calcitonin levels. The pentagastrin stimulation test was performed in the morning either before the hemodialysis session or on the dialysis-free day. In patients treated with peritoneal dialysis, the test was done in the morning after the first change of the bag. The thyroid gland was examined by palpation and/or ultrasound in every patient who received pentagastrin stimulation.

Measurements

Blood chemistry (serum calcium, phosphorus, and creatinine) was determined in a clinical laboratory at the Medical University Vienna. Overall, renal function (estimated glomerular filtration rate; ml/min/1.73 m^2) was estimated from serum creatinine levels using the short modification of diet in renal disease formula[19] to classify

CKD stages.[20] Intact parathyroid hormone serum level was determined by a commercially available Radio immuno assay (Nichols Institute Diagnostics, San Clemente, CA, USA).

During the time of this study, three assays were used for measurement of calcitonin serum concentrations.[21] All three assays used monoclonal mouse antibodies: 1. January 1997 – May 1999: Calcitonin immunoradiometric assay (CIS Bio International, Gif-Sur-Yvette, France) (analytical sensitivity: 1–4 pg/ml; coefficient of variation: 3–15%) is using two monoclonal mouse antibodies (reference interval: females < 10 pg/ml; males < 10 pg/ml). 2. June 1999 – March 2000: Medgenix CT-U.S.-IRMA kit (BioSource Europe S.A., Nivelles, Belgium) is an immunoradiometric assay (analytical sensitivity: 1–2 pg/ml; coefficient of variation: 3–12%) using two monoclonal mouse antibodies (reference interval: females < 10 pg/ml; males < 10 pg/ml). 3. April 2000 – December 2004: Nichols Advantage Calcitonin Assay (Nichols Institute Diagnostics, San Clemente, CA, USA) is an immunochemiluminometric assay (analytical sensitivity: 1 pg/ml; coefficient of variation: 8%) using two monoclonal mouse antibodies (reference interval: females < 4.6 pg/ml; males < 11.5 pg/ml). Independently from the differences in the reference intervals of the three assays used, a pentagastrin test was recommended when basal calcitonin concentrations exceeded 10 pg/ml.[15] The upper limit of normal for pentagastrin-stimulated calcitonin in 19 healthy volunteers was found to be 43.0 pg/ml for males and 36.8 pg/ml for females.[22]

Total thyroidectomy

After dissection of both recurrent nerves, a systematic microdissection of the lymph node compartment from the upper thoracic outlet up to the larynx was performed. In patients with tertiary hyperparathyroidism, a total parathyroidectomy with immediate autotransplantation or subtotal parathyroidectomy with removal of three and a half glands including transcervical thymectomy and cryopreservation of parathyroid tissue was performed.[2]

Histology

Surgical specimens from total thyroidectomy and lymphadenectomy by modified radical neck dissections were blocked entirely. Tissue was processed by standard methods, paraffin embedded and cut in sections of 5 μm. All specimens were stained with hematoxylin and eosin, and calcitonin immunostaining was performed using the avidin–biotin–peroxidase technique with a commercially available antibody (Chemicon, Temuecula, CA, USA).[23] C-cell hyperplasia was diagnosed when at least one field at an $\times 100$ magnification contained more than 50 C cells in each thyroid lobe.[24] Medullary thyroid carcinoma and papillary thyroid carcinoma were diagnosed[24] and staged according to the International Union of Cancer TNM classification (5th edition, 1997).

Statistical analysis

Continuous data are summarized as medians and full ranges, categorical data are described by counts and frequencies. We compared basal and stimulated calcitonin serum concentrations of patients with medullary thyroid carcinoma and of patients with C-cell hyperplasia by Mann–Whitney-*U* test.

We used ROC analysis to describe the performance of basal and pentagastrin-stimulated calcitonin levels for differentiating medullary thyroid carcinoma and C-cell hyperplasia. The area under the ROC plot was calculated as an index of diagnostic accuracy. The variability of sensitivity/specifity estimates is indicated by 95% exact confidence intervals. SAS (Version 9.1, 2002–2003, SAS Institute Inc.) and SPSS (Version 12.0, 2003, SPSS Inc.) were used for all analyses.

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