Utility of Serum Procalcitonin for Screening and Risk Stratification of Medullary Thyroid Cancer

Andreas Machens, Kerstin Lorenz, and Henning Dralle

Department of General, Visceral, and Vascular Surgery, Martin Luther University Halle-Wittenberg, D-06097 Halle (Saale), Germany

Context: The clinical utility of procalcitonin has not been demonstrated across the whole spectrum of medullary thyroid cancer (MTC).

Objective: This serum biomarker validation study aimed at defining the diagnostic accuracy of procalcitonin for screening and risk stratification of MTC and delineating biochemical thresholds predictive of lymph node involvement in the neck and mediastinum.

Design and Setting: This was a retrospective analysis at a tertiary referral center.

Patients: Included in this study were 457 consecutive patients with previously untreated MTC, 112 of whom had procalcitonin and calcitonin serum levels determined before the initial operation.

Intervention: The intervention was compartment-oriented surgery.

Main Outcome Measures: Main outcome measures included primary tumor diameter, extrathyroidal extension, lymph node metastases, distant metastases, and biochemical cure.

Results: Receiver operating characteristics analyses revealed similar diagnostic accuracy for procalcitonin vs the current calcitonin standard, yielding comparable areas under the curve for primary tumors at thresholds of 10 (0.94 vs 0.93) and 40 (0.92 vs 0.84) mm; extrathyroidal extension (0.84 vs 0.83), lymph node metastasis (0.88 vs 0.86), and distant metastasis (0.93 vs 0.91). Lymph node metastases were present in the ipsilateral lateral neck with procalcitonin levels $\leq$1.0 ng/mL and the ipsilateral central neck with procalcitonin levels $\leq$0.25 ng/mL. Above a threshold of 1.0 ng/mL, lymph node metastases emerged in the contralateral central and lateral neck and above 5.0 ng/mL also in the upper mediastinum. When procalcitonin levels exceeded 1, 5, 10, and 50 ng/mL, biochemical cure rates declined to no more than 71%, 36%, 23%, and 10%, respectively.

Conclusion: Serum procalcitonin, having comparable diagnostic accuracy, has great potential to replace serum calcitonin as a new standard of care in the management of MTC because it does not need to be kept cool on ice or frozen and is easier to manage at the community level. **(*J Clin Endocrinol Metab* 99: 2986–2994, 2014)**

Owing to their neural crest derivation, medullary thyroid cancer (MTC) cells synthesize, store, and release neuroendocrine substances. Conceptually, any of these peptides would qualify as a biomarker candidate of MTC: calcitonin gene-related peptide, calcitonin, procalcitonin, chromogranin A and B, and/or synaptophysin (1, 2). The technology to measure serum calcitonin, evolving over 4 decades, has been pivotal in delineating MTC as a tumor entity in its own right, setting it apart from follicular-cell thyroid cancers (3). Biochemical screening programs for MTC, capitalizing on these advances in diagnostic assay development, have been founded on the determination of calcitonin in peripheral venous blood, from which the term C-cell (C denoting calcitonin) cancer

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received January 29, 2014. Accepted May 1, 2014.
First Published Online May 19, 2014

Abbreviations: CI, confidence interval; MTC, medullary thyroid cancer.

takes its name. Calcitonin, with or without iv stimulation with pentagastrin and/or calcium, has been used for screening, biochemical diagnosis, outcome evaluation, or prognostic stratification of patients (4–7).

Many limitations compromise the utility of calcitonin for biochemical monitoring in patients with C-cell disease. Typically, the serum levels of calcitonin may vary markedly during the day, reflecting the pulsatile secretion of the hormone. Calcitonin, a 32-amino-acid monomeric peptide, results from cleavage and posttranslational processing of procalcitonin. Calcitonin comes in several immunoreactive isoforms and fragments, giving rise to disparate assay formats and calcitonin antibody concentrations (8). Commonly, different calcitonin assays produce disparate results for the same blood sample, rendering it challenging to compare calcitonin measurements obtained with different assays (9). Assay-specific variations made it difficult to set uniform clinical calcitonin thresholds that apply around the globe. To complicate the matter, calcitonin has a concentration-dependent biphasic half-life of ~15 and ~40 minutes at physiological conditions and ~3 and ~30 hours at elevated concentrations (8). Adding another layer of complexity, calcitonin is so unstable that it is rapidly degraded by serum proteases at room temperature. Even under conditions of refrigeration, calcitonin decays by 23% after 12 hours, by 35% after 24 hours, and by 65% after 7 days, causing false low test results (8, 9). In clinical practice, the need for expedited processing of blood samples that must be kept on ice or frozen perhaps is the greatest disadvantage of calcitonin screening programs, hampering wider use of this otherwise highly sensitive tumor marker outside specialist settings.

Largely devoid of these problems is procalcitonin, the precursor peptide of calcitonin derived from preprocalcitonin. In healthy individuals, procalcitonin, a 116-amino-acid peptide, originates from the parafollicular C-cells of the thyroid gland. Pentagastrin stimulation leads to no more than marginal increases in procalcitonin serum levels (9). Unlike calcitonin, procalcitonin is a very stable protein featuring a concentration-independent in vivo half-life of 20 to 24 hours (8). Previous studies, comparing the abilities of procalcitonin and calcitonin to distinguish MTC from other conditions, revealed comparable utility (8, 9). Using the same antibodies, all commercial procalcitonin assays yield similar results. This universal comparability of procalcitonin measurements would enable worldwide standardization of biomarker-based diagnosis, therapy, and monitoring of patients with MTC (9).

Before becoming an adjunct to, or replacing, calcitonin as a new standard of care, procalcitonin needs to demonstrate at least comparable utility across the whole spectrum of MTC using standardized time points and exact tumor staging. The present investigation of patients with MTC was undertaken to compare the clinical utility of procalcitonin and calcitonin for screening and initial risk stratification and define procalcitonin thresholds predictive of lymph node metastases to the central and lateral neck and the upper mediastinum.

Patients and Methods

Patients

A total of 886 consecutive patients (457 patients with untreated tumors and 429 patients with recurrent or persistent tumors) underwent operations for MTC at this institution between November 1994 and February 2014. Included in this study were all 457 patients with untreated MTC. Chart review identified a subset of 112 patients who had both procalcitonin and calcitonin serum levels taken before the initial operation. These 112 patients, undergoing elective procedures, were free of infections at the time of the operation. Patients with sporadic and hereditary disease, faring the same after adjustment for extent of disease (10), were evaluated as one group.

Measurements of procalcitonin and calcitonin serum levels

Procalcitonin

All procalcitonin serum levels were determined on a Kryptor system (Brahms; Thermo Scientific) set up in February 2000 at the university hospital. The Brahms PCT Kryptor assay, which has remained unchanged ever since, measures procalcitonin concentrations between 0.02 and 5000 ng/mL (0.02–50 ng/mL directly and up to 5000 ng/mL after sample dilution). Based on the manufacturer's package insert, the intra-assay coefficient of variation and the interassay coefficient of variation are 2% to 3% on the whole procalcitonin concentration range. The functional assay sensitivity (defined as the lowest analyte concentration that can be determined with an interassay coefficient of variance <20%) is given as 0.06 ng/mL.

Calcitonin

Serum levels of calcitonin were measured until May 2004 with the ELSA-hCT solid 2-site immunoradiometric calcitonin assay (CIS bio international; normal range <10 pg/mL) (4). After May 2004, the Immulite 2000 automated calcitonin assay (Diagnostic Products Corporation), enabling determination of calcitonin levels immediately before surgery, replaced the ELSA-hCT assay (normal range of the Immulite 2000 assay is <5 pg/mL for women and <8.4 pg/mL for men). The Immulite 2000 and ELSA-hCT calcitonin assays are both linearly related to the Nichols-Advantage assay (11, 12).

Biochemical cure

Postoperative normalization of serum calcitonin (biochemical cure) was assumed when the upper normal limit of the respective calcitonin assay (10 pg/mL for either gender and <5 pg/mL for women or <8.4 pg/mL for men, respectively) was not exceeded basally.

Total thyroidectomy and compartment-oriented surgery

All 457 patients but 1 had a total thyroidectomy. In lieu of planned thyroidectomy, this exceptional patient, who was biochemically cured because his 19-mm large sporadic MTC was confined to the thyroid gland, underwent lobectomy with subtotal thyroid resection because intraoperative nerve monitoring suggested the possibility of injury to the recurrent laryngeal nerve on the side of completion. Based on the principles laid out elsewhere (4), lymph node dissections had been performed in addition, using the compartment-oriented approach (13) with optical magnification and bipolar coagulation (14) in the central neck compartment (409 patients; 89.5%), the lateral neck compartment ipsilateral to (332 patients; 72.6%) and contralateral to the largest primary thyroid tumor (308 patients; 67.4%), and the upper mediastinum via complete median sternotomy (48 patients; 10.5%). Informed consent was obtained before each operation that represented standard practice of care according to the practice guidelines of the German Cancer Association (15). Distant metastases were not an exclusion criterion because of the longevity of patients with metastatic MTC.

Histopathological examination and tumor staging

All specimens were subjected to histopathologic examination and embedded in paraffin. Hematoxylin and eosin staining and calcitonin immunohistochemistry were performed throughout. MTC was diagnosed on evidence of tumor extension beyond the basement membrane, demonstration of lymphatic or vascular invasion, or a combination thereof based on the World Health Organization's *International Histological Classification of Tumors* (16, 17). Primary tumor diameter was ascertained by direct measurements on the thyroid specimens. When multiple MTCs were present, only the largest primary tumor was considered. Only histopathologically involved nodes were counted as lymph node metastases, whereas undissected (clinically unsuspicious) and negative nodes were deemed to be free of lymph node metastasis for the purpose of this study. This conservative approach yielded minimum estimates of lymph node involvement. The diagnosis of distant metastasis was based on radiological evidence on ultrasonography, computed tomography, magnetic resonance imaging, 18-fluorodeoxyglucose or 18-fluoro-dopa positron emission tomography, or any combination thereof.

Data analysis

Categorical and continuous data were tested on univariate analysis using the two-tailed Fisher's exact test and one-way ANOVA, respectively. Spearman's rank correlation coefficient was calculated to evaluate correlations between incremental brackets of primary tumor diameter (≤5, 5.1–10, 10.1–20, 20.1–40, and >40 mm) or lymph node metastases (0, 1–5, 6–20, and >20 nodes), considering established thresholds (4) and the number of patients available for analysis, and corresponding preoperative procalcitonin and calcitonin serum levels and procalcitonin to calcitonin ratios. A similar stratification strategy was pursued to assess correlations between incremental brackets of preoperative serum procalcitonin (≤0.10, 0.11–0.25, 0.26–0.50, 0.51–1.0, 1.01–5.0, 5.01–10.0, 10.01–50.0, and >50 ng/mL) and largest primary tumor diameter, frequency, number, and pattern of lymph node metastases, distant metastasis, corresponding calcitonin serum levels and procalcitonin to calcitonin ratios, and biochemical cure.

Multiple testing was corrected for with the Bonferroni method as appropriate. Suitability for prediction of larger primary tumors, extrathyroidal extension, lymph node metastases, and distant metastasis was determined using receiver operating characteristics analysis, with the area under the curve representing a summary measure of accuracy. The level of statistical significance (all values were two-tailed) was set at $P < .05$.

Results

Patient characteristics by availability of preoperative serum levels of procalcitonin

All 457 patients were categorized by the availability (112 patients) or unavailability (345 patients) of procalcitonin serum levels before the initial thyroid operation (Table 1). With few exceptions, the 2 patient subgroups revealed comparable clinical and biochemical characteristics. Patients without available procalcitonin serum levels were more often *RET* gene carriers (45% vs 19%; $P < .001$), were almost 8 years younger (means of 43.4 vs 51.3 years; $P = .001$), and harbored smaller thyroid primaries (means of 14.2 vs 19.2 mm; $P = .005$). The first 2 associations remained statistically significant after correction for multiple testing.

By implication, more patients with sporadic and clinically apparent disease, having a greater chance of harboring MTC, underwent biochemical screening for procalcitonin during the work-up for MTC.

Comparison of preoperative serum levels of procalcitonin and calcitonin

To enable direct comparisons between corresponding serum levels of procalcitonin and calcitonin, primary tumor diameter (≤5, 5.1–10, 10.1–20, 20.1–40, and >40 mm) and lymph node metastases (0, 1–5, 6–20, and >20 nodes) were categorized according to the number of patients available for analysis (Table 2). Notably, the individual correlations of these 2 biomarkers with primary tumor diameter and the number of lymph node metastases were equally strong, resulting in correlation coefficients of 0.80 (procalcitonin) and 0.82 (calcitonin) and 0.70 (procalcitonin) and 0.65 (calcitonin), respectively. No statistical significance was seen for the corresponding procalcitonin to calcitonin ratio (Table 2; mean ratio 7.1; 95% confidence interval [CI] 5.2–9.0).

Receiver operating characteristics analyses revealed similar diagnostic accuracy for procalcitonin vs the current calcitonin standard in MTC, yielding comparable areas under the curve (with 95% CIs) for primary tumors at thresholds of 10 mm (0.94 [0.90–0.99] vs 0.93 [0.88–0.97]; Figure 1A) and 40 mm (0.92 [0.86–0.98] vs 0.84 [0.75–0.94]; Figure 1B), extrathyroidal extension (0.84 [0.76–0.92] vs 0.83 [0.74–0.91]; Figure 1C), lymph node metastasis (0.88 [0.82–0.95] vs 0.86 [0.79–0.93]; Figure 1D), and distant metastasis (0.93 [0.87–0.98] vs 0.91 [0.84–0.97]; Figure 1E). These results were almost identical when the receiver

Table 1. Clinical and Biochemical Patient Characteristics by Availability of Procalcitonin Serum Levels Before the Initial Thyroid Operation[a]

Variable	Procalcitonin Serum Level Before Initial Thyroidectomy		*P*
	Available (112 patients)	Unavailable (345 patients)	
Patient age at tissue diagnosis, y	51.3 (48.1–54.6)	43.4 (41.0–45.7)	.001[b]
Gender, male	59 (53)	152 (44)	.13
RET gene carriers	21 (19)	154 (45)	<.001[b]
Preoperative basal calcitonin level, pg/mL	3350 (1637–5063)	3796[c] (2268–5324)	.75
Largest primary tumor diameter, mm[d]	19.2 (15.7–22.7)	14.2 (12.6–15.8)	.005
Extrathyroidal tumor extension	25 (22)	62 (18)	.33
Lymph node involvement	52 (46)	129 (37)	.10
Number of lymph node metastases[e]	5.8 (3.7–7.8)	7.2 (5.4–9.0)	.39
Number of removed lymph nodes[e]	58.1 (52.5–63.7)	53.3 (49.2–57.4)	.23
Distant organ involvement	11 (10)	40 (12)	.73
Biochemical cure (normalization of calcitonin levels)[f]	68 (65)	216 (68)	.55

[a] Results are shown as mean (95% CI) or n (%).

[b] Statistically significant after Bonferroni correction for multiple testing.

[c] Based on 329 patients with pertinent information.

[d] Based on 109 and 330 patients with pertinent information.

[e] Based on 110 and 317 patients with systematic lymph node dissection.

[f] Based on 105 and 318 patients with pertinent information.

operating characteristics analyses were restricted to the period in which calcitonin was measured exclusively with the Immulite 2000 assay (data not shown).

Extent of disease, calcitonin levels, and biochemical cure by serum level of procalcitonin

To characterize their utility for screening and risk stratification of MTC, preoperative procalcitonin serum levels were correlated with largest primary tumor diameter, number of lymph node metastases, the corresponding preoperative basal calcitonin serum levels, and procalcitonin to calcitonin ratios, and biochemical cure rates. As depicted in Table 3, higher procalcitonin levels signified larger primary tumors and greater numbers of lymph node metastases. Incremental brackets of preoperative procalcitonin levels, reflecting extent of disease, correlated closely with the preoperative calcitonin serum level ($r = 0.88$), the largest primary tumor diameter ($r = 0.79$), and the number of lymph node metastases ($r = 0.68$), with the procalcitonin to calcitonin ratio ($r = 0.19$) lagging behind. By implication, the categories of procalcitonin levels examined explained 77% (0.88^2), 62% (0.79^2), 46% (0.68^2), and 4% (0.19^2) of the respective

Table 2. Comparison of Preoperative Serum Levels of Procalcitonin and Calcitonin by Primary Tumor Diameter and Number of Lymph Node Metastases

Variable	n	Basal Serum Levels Before Initial Thyroidectomy							
		Procalcitonin (ng/mL)			Basal Calcitonin (pg/mL)			Procalcitonin (pg/mL) to Basal Calcitonin (pg/mL) Ratio	
		Mean (95% CI)	*P*	$r_{Spearman}$	Mean (95% CI)	*P*	$r_{Spearman}$	Mean (95% CI)	*P*
Largest primary tumor diameter, mm[a]			<.001[b]	0.80		<.001[b]	0.82		.88
≤5	18	0.3 (0–0.6)			107 (0–264)			7.0 (4.9–9.1)	
5.1–10	25	0.9 (0.4–1.4)			235 (162–308)			4.6 (1.7–7.4)	
10.1–20	31	8.4 (2.2–14.6)			2013 (331–3695)			7.6 (4.1–11.0)	
20.1–40	25	24.7 (8.6–40.4)			7785 (1104–14,467)			5.7 (2.0–9.4)	
>40	10	95.4 (0–1971)			9468 (2053–16 883)			17.0 (2.4–31.6)	
Number of lymph node metastases[c]			<.001[b]	0.70		<.001[b]	0.65		.35
0	59	1.5 (0.8–2.3)			436 (261–611)			5.1 (3.7–6.5)	
1–5	22	8.0 (3.4–12.6)			2609 (929–4289)			6.5 (2.3–10.8)	
6–20	18	34.5 (14.9–64.0)			6917 (2524–11 309)			9.1 (3.9–14.3)	
>20	11	41.9 (11.5–72.2)			14,543 (0–30 275)			10.7 (0.8–20.5)	

[a] Excluding 3 patients without pertinent data due to extrathyroidal tumor extension.

[b] Statistically significant after Bonferroni correction for multiple testing.

[c] Excluding 1 patient with a 0.8 mm large primary tumor and 1 patient with bone and liver metastases who did not have lymph node dissection.

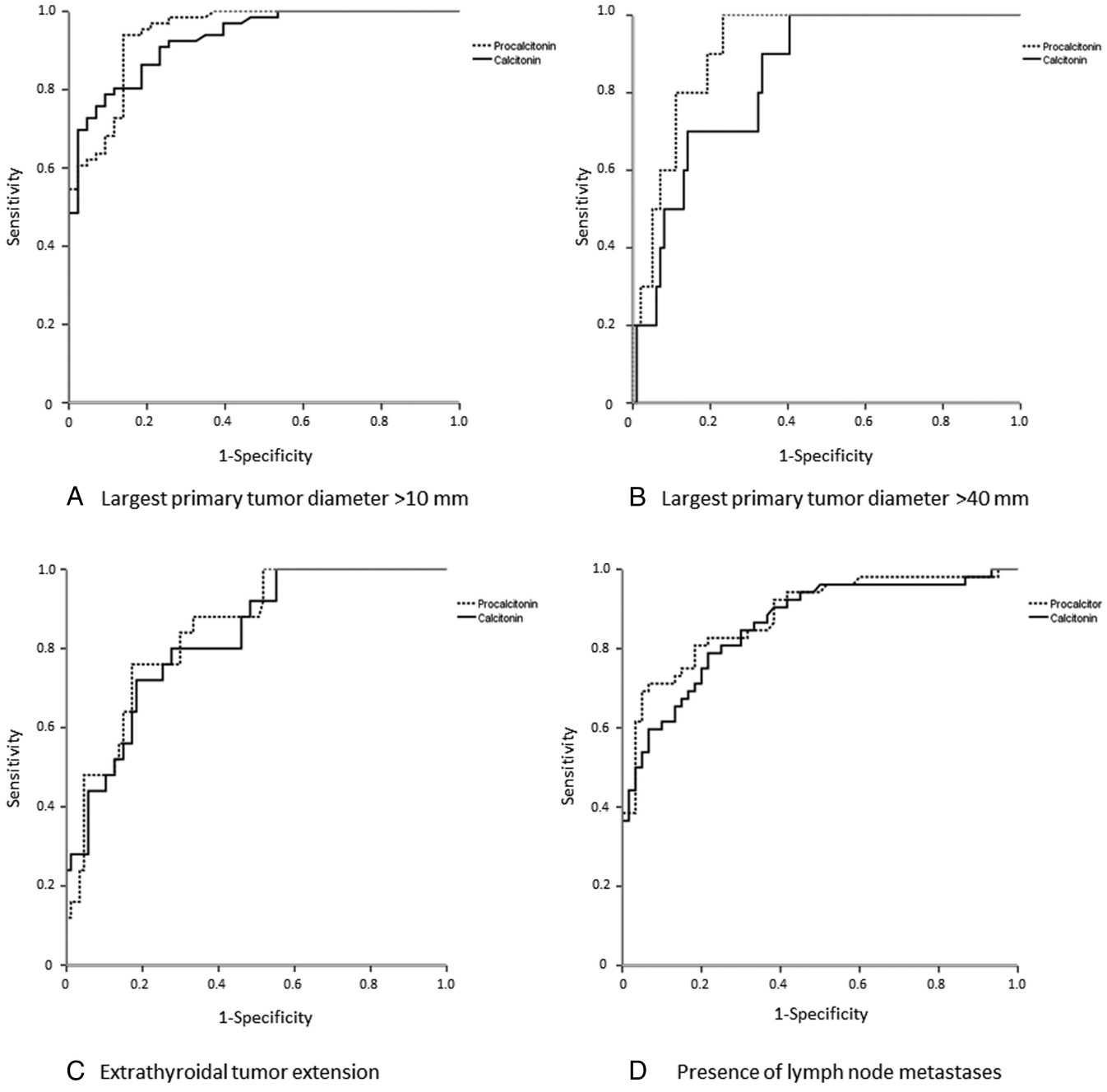
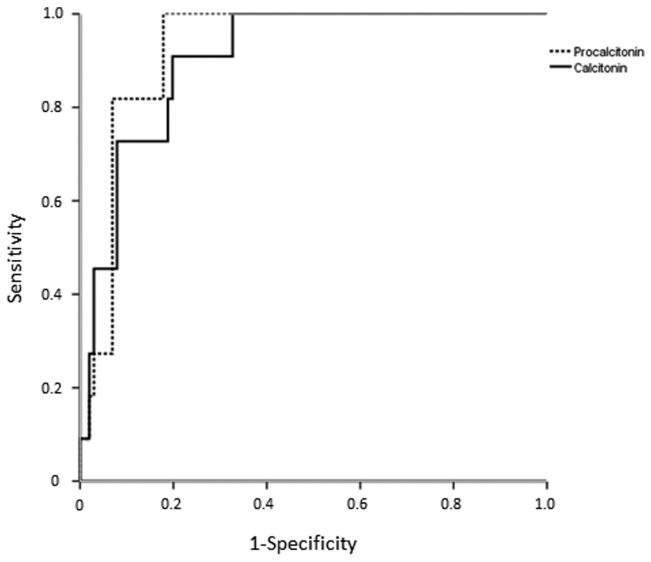


Figure 1. Receiver operating characteristics analysis comparing procalcitonin with calcitonin serum levels.

variation in corresponding basal calcitonin levels, primary tumor diameter, the number of lymph node metastases, and the procalcitonin to calcitonin ratio. Conversely, when procalcitonin levels crossed thresholds of 1, 5, 10, and 50 ng/mL, biochemical cure rates declined to no more than 71%, 36%, 23%, and 10%, respectively (Table 3).

Frequency and pattern of lymph node metastases by serum level of procalcitonin

From a surgical perspective, the probability of involvement of a specific lymph node compartment can be more important than the total number of lymph node metastases. Table 4 provides a breakdown by procalcitonin level of the minimum involvement of the central and lateral neck and the upper anterior mediastinum and lymph node and distant metastasis. There was a statistically significant ($P \leq .005$) association between rising procalcitonin serum levels and increasing rates of lymph node metastases overall and in the central and lateral neck and upper mediastinum. As long as preoperative procalcitonin serum levels did not exceed thresholds of 0.25 and 1.0 ng/mL, respectively, lymph node metastases were limited to the ipsilateral central and lateral neck. Once they surpassed a threshold of 1.0 ng/mL, lymph node metastases began appearing in the contralateral central



E Presence of distant metastases

Figure 1. Continued.

and lateral neck, and above a threshold of 5.0 ng/mL also in the upper mediastinum, paralleling the presence of distant metastasis (Table 4).

Extent of disease with preoperative procalcitonin serum levels ≤0.15 ng/mL

Occult MTC, denoting thyroid primaries no greater than 10 mm, may occur with a serum procalcitonin of ≤0.15 ng/mL (Table 5). Indeed, 13 (11.6%) of our 112 patients with MTC displayed preoperative procalcitonin serum levels ≤0.15 ng/mL. The lowest procalcitonin level in the presence of MTC was 0.07 ng/mL. In 2 of our 13 patients, a 23-year-old female and a 32-year-old male gene carrier from 2 V804M *RET* families, the initial thyroid operation had been triggered by the positive DNA test and carrier age, not the biochemical test results. Remarkably, 1 of our 11 patients with sporadic MTC, having a serum procalcitonin as low as 0.09 ng/mL, had lymph node metastasis, affecting 1 of 14 dissected nodes.

Discussion

This investigation of 112 patients with MTC, using standardized time points (before the initial operation) and exact tumor staging, validates previous suggestions that serum procalcitonin is a clinically important biomarker of MTC comparable in diagnostic accuracy to basal serum calcitonin. Serum procalcitonin beyond being a diagnostic biomarker of MTC as previously suggested, is also a powerful predictive biomarker enabling initial risk stratification of MTC.

Procalcitonin screening for MTC

Nonmedullary thyroid diseases are linked to serum levels of procalcitonin <0.1 ng/mL (18). False-positive test results are rated at 0.5% (19). To exclude occult MTC, various procalcitonin thresholds have been proposed: 0.05 ng/mL disregarding C-cell hyperplasia (20); 0.10 ng/mL (18), 0.15 ng/mL (8), and 0.16 ng/mL considering C-cell hyperplasia (20); and <0.25 ng/mL to exclude MTC (9).

In this series, procalcitonin serum levels as low as 0.07 and 0.09 ng/mL were connected to node-negative and node-positive MTC. This finding hints at an overlap between MTC and other conditions around the 0.1 ng/mL mark, necessitating a trade-off between undertreatment (if expectant observation is opted for in the presence of MTC) and potential overtreatment (if thyroidectomy is per-

Table 3. Extent of Disease, Calcitonin Levels, and Biochemical Cure by Serum Level of Procalcitonin

		Extent of Disease, Mean (95% CI)		Biochemistry Before Initial Thyroidectomy, Mean (95% CI)		
Procalcitonin Serum Level Before Initial Thyroidectomy, ng/mL	Patients, n	Largest Primary Tumor Diameter (mm)[a]	Number of Lymph Node Metastases[c]	Basal Calcitonin Serum Level (pg/mL)	Procalcitonin (pg/mL) to Basal Calcitonin (pg/mL) Ratio	Biochemical Cure,[b] n (%)
≤0.10	5	2.0 (0.4–3.5)	0.3 (0–1.1)	11 (7–16)	7.8 (4.7–10.8)	5 (100)
0.11–0.25	17	5.8 (4.5–7.2)	0	107 (40–174)	4.0 (2.1–5.9)	14 (88)
0.26–0.50	13	8.8 (6.2–11.3)	0.2 (0–0.6)	132 (69–194)	4.9 (2.4–7.5)	12 (92)
0.51–1.0	13	16.7 (11.0–22.4)	2.8 (0–6.6)	649 (198–1100)	2.4 (1.5–3.3)	12 (92)
1.01–5.0	28	16.2 (13.6–18.8)	3.3 (0.9–5.7)	913 (462–1363)	6.0 (3.5–8.6)	17 (71)
5.01–10.0	11	24.5 (16.4–32.7)	9.9 (2.8–17.0)	3134 (230–6038)	6.4 (2.4–10.5)	4 (36)
10.1–50.0	14	40.1 (19.3–60.9)	16.2 (4.7–27.8)	8144 (4152–12,135)	8.7 (1.4–16.0)	3 (23)
>50	11	42.7 (30.7–54.7)	16.7 (10.6–22.8)	17,192 (1616–32,768)	21.1 (7.4–34.9)	1 (10)
$r_{Spearman}$		0.79	0.68	0.88	0.19	
P		<.001[d]	<.001[d]	<.001[d]	.04	<.001

[a] Excluding 3 patients without pertinent data due to extrathyroidal tumor extension.

[b] Excluding 7 patients without pertinent information.

[c] Excluding 1 patient with a 0.8-mm large primary tumor and 1 patient with bone and liver metastases who did not have lymph node dissection.

[d] Statistically significant after Bonferroni correction for multiple testing.

Table 4. Frequency and Pattern of Lymph Node Metastases by Serum Level of Procalcitonin

Procalcitonin Serum Level Before Initial Thyroidectomy, ng/mL	Patients, n	Any Lymph Node Metastasis, n (%)	Ipsilateral Neck, n (%)		Contralateral Neck, n (%)		Upper Mediastinum, n (%)	Distant Metastasis, n (%)
			Central	Lateral	Central	Lateral		
≤0.10	5	1 (20)	1 (20)	0	0	0	0	0
0.11–0.25	17	0	0	0	0	0	0	0
0.26–0.50	13	2 (15)	1 (8)	1 (8)	0	0	0	0
0.51–1.0	13	5 (38)	5 (38)	3 (23)	0	0	0	0
1.01–5.0	28	11 (39)	10 (36)	7 (25)	4 (14)	1 (4)	0	0
5.01–10.0	11	10 (91)	6 (55)	8 (73)	5 (45)	4 (36)	1 (9)	2 (18)
10.1–50.0	14	12 (86)	7 (50)	10 (71)	6 (43)	6 (43)	3 (21)	5 (36)
>50	11	11 (100)	7 (64)	10 (91)	6 (55)	4 (36)	3 (27)	4 (36)
Total	112	52 (46)	37 (33)	39 (35)	21 (19)	15 (13)	7 (6)	11 (10)
P		<.001	<.001	<.001	<.001	<.001	.005	<.001

formed for conditions that would not warrant an operation in the absence of MTC). Such overlap between MTC and other thyroid disease has also been observed around the 20 pg/mL threshold of calcitonin, below which occult MTC can occur (21). In clinical practice, a value judgment is necessary, to the effect that lowering the optimal biochemical cutoff point is lowered the more the greater is the a priori probability of MTC, the utility gain from the surgical intervention, and the risk aversion.

Strengths of the study

This is the largest, most comprehensive analysis of the utility of serum procalcitonin for screening and risk stratification of MTC reported to date. All our 112 institutional MTC patients had procalcitonin and basal calcitonin serum levels measured at the same time before the initial thyroid operation, enabling direct comparisons between the 2 biomarkers in a standardized environment. To avoid confounding by the extent of earlier surgery, only patients with untreated MTC were considered in this study. All other series included mostly, or exclusively, patients who had already been operated on for MTC (9, 20, 22). In these series, the postoperative blood samples for determination of procalcitonin and basal calcitonin serum levels had been taken at ill-defined time points during follow-up, precluding any standardization. To compensate for the rarity of the disease, at times, patients from more than one institution were pooled (20), at the expense of introducing more heterogeneity into the combined series. This disparity of study designs may explain the greater correlation between procalcitonin and basal calcitonin levels in our series: $r = 0.88$, as opposed to $r = 0.61–0.74$ elsewhere (9, 22). This correlation between procalcitonin and calcitonin, which reportedly is stronger in patients with active MTC ($r = 0.95$) than in patients with MTC in remission ($r = 0.60$), disappears in patients with inflammatory disease (23).

It may be interesting to note that patients with persistent MTC after neck surgery are thought, based on molecular weights of 12 740 for procalcitonin and 3422 for calcitonin and a procalcitonin to calcitonin ratio of 7.6, to secrete approximately 1.9 molecules of procalcitonin for 1 molecule of calcitonin (22). As demonstrated in this study, this ratio is not stable across the disease spectrum. It enlarged with more advanced disease (primary tumors

Table 5. Extent of Disease in the 13 Patients With Preoperative Procalcitonin Serum Levels ≤0.15 ng/mL

Biochemistry		Demographics		Extent of Disease		
Procalcitonin Level, ng/mL	Basal Calcitonin Level, pg/mL	Age, y	Gender	Primary Tumor Diameter, mm	Involved/Removed Nodes, n/N	Biochemical Cure
0.07	7.8	32[a]	Male	2	0/1	Yes
0.07	14.7	47	Female	1 and 0.3	0/18	Yes
0.08	7.2	23[a]	Female	2	0/2	Yes
0.09	14.3	68	Female	4	1/14	Yes
0.10	13.2	66	Male	0.8	N/A	Yes
0.12	33.8	59	Male	3	0/41	Yes
0.12	164	45	Female	10	0/67	Yes
0.12	495	64	Male	6	0/26	Yes
0.13	9.7	65	Male	2	0/50	Yes
0.13	65.1	41	Female	4	0/1	Yes
0.14	61.5	63	Female	9	0/69	Yes
0.14	66.1	30	Female	5	0/25	Yes
0.15	15.8	56	Female	4	0/54	No

[a] Asymptomatic carrier from a V804M *RET* family identified through DNA-based screening.

in excess of 40 mm and/or 6 lymph node metastases; Table 2), which may reflect dedifferentiation.

Limitations of the study

This single-center study shares those limitations inherent in a retrospective study. First, *RET* gene carriers, harboring more frequently occult thyroid primaries, were underrepresented in the absence of clinical evidence of MTC (Table 1) because it was precisely that confirmation that prompted determination of preoperative procalcitonin serum levels. To minimize the potential impact of such underrepresentation, stratification by incremental brackets of procalcitonin serum levels, primary tumor diameter, and numbers of lymph node metastases was employed throughout. In a rare disease, issues of statistical power can pose difficulties, even in a population of more than 100 patients. In tumor marker prognostic studies, significance levels due to multiple testing, subset analyses, and cutoff optimization always are issues (24). This is why correction for multiple testing was used throughout, abstaining from subset analyses and dichotomization of thresholds in favor of more descriptive analyses. Because the serum level of calcitonin influences the work-up to establish the diagnosis of MTC, the clinical performance of calcitonin is biased toward 100% sensitivity. This methodological limitation, also dubbed inclusion bias or work-up bias, raises the bar such that procalcitonin cannot achieve superiority over the current calcitonin standard (8).

Future perspectives

Prognostic validation of serum procalcitonin as an emerging standard of biochemical screening is relatively straightforward. Although the gold standard for predictive marker validation continues to be a prospective randomized clinical trial, retrospective validation can be acceptable as a marker strategy (25), in particular for a rare disease. Serum procalcitonin provides substantial added value to the current practice of calcitonin-based screening and risk stratification of MTC. Anecdotal evidence also suggests that some MTCs preferentially secrete procalcitonin, the monitoring of which is becoming a method of choice in tumors failing to secrete calcitonin adequately (26). Unlike calcitonin, procalcitonin does not need to be kept cool on ice or frozen during the entire process chain and is easier to manage at the community level, giving it great potential to replace calcitonin as a new standard of care in the management of MTC.

Acknowledgments

Address all correspondence and requests for reprints to: Andreas Machens, MD, Department of General, Visceral, and Vascular Surgery, Martin Luther University Halle-Wittenberg, Ernst-Grube-Strasse 40, D-06097 Halle (Saale), Germany. E-mail: AndreasMachens@aol.com.

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

Disclosure Summary: The authors indicated no potential conflicts of interests.

References

1. **Camacho CP, Lindsey SC, Melo MC, et al.** Measurement of calcitonin and calcitonin gene-related peptide mRNA refines the management of patients with medullary thyroid cancer and may replace calcitonin-stimulation tests. *Thyroid*. 2013;23:308–316.
2. **Frank-Raue K, Machens A, Leidig-Bruckner G, et al.** Prevalence and clinical spectrum of nonsecretory medullary thyroid carcinoma in a series of 839 patients with sporadic medullary thyroid carcinoma. *Thyroid*. 2013;23:294–300.
3. **Tashjian AH Jr, Melvin EW.** Medullary carcinoma of the thyroid gland. Studies of thyrocalcitonin in plasma and tumor extracts. *N Engl J Med*. 1968;279:279–283.
4. **Machens A, Dralle H.** Biomarker-based risk stratification for previously untreated medullary thyroid cancer. *J Clin Endocrinol Metab*. 2010;95:2655–2663.
5. **Yip DT, Hassan M, Pazaitou-Panayiotou K, et al.** Preoperative basal calcitonin and tumor stage correlate with postoperative calcitonin normalization in patients undergoing initial surgical management of medullary thyroid carcinoma. *Surgery*. 2011;150:1168–1177.
6. **Machens A, Dralle H.** Prognostic impact of N staging in 715 medullary thyroid cancer patients: proposal for a revised staging system. *Ann Surg*. 2013;257:323–329.
7. **Machens A, Dralle H.** Benefit-risk balance of reoperation for persistent medullary thyroid cancer. *Ann Surg*. 2013;257:751–757.
8. **Algeciras-Schimnich A, Preissner CM, Theobald JP, Finseth MS, Grebe SK.** Procalcitonin: a marker for the diagnosis and follow-up of patients with medullary thyroid carcinoma. *J Clin Endocrinol Metab*. 2009;94:861–868.
9. **Kratzsch J, Petzold A, Raue F, et al.** Basal and stimulated calcitonin and procalcitonin by various assays in patients with and without medullary thyroid cancer. *Clin Chem*. 2011;57:467–474.
10. **Machens A, Schneyer U, Holzhausen HJ, Dralle H.** Prospects of remission in medullary thyroid carcinoma according to basal calcitonin level. *J Clin Endocrinol Metab* 2005;90:2029–2034.
11. **Bieglmayer C, Vierhapper H, Dudczak R, Niederle B.** Measurement of calcitonin by immunoassay analyzers. *Clin Chem Lab Med*. 2007; 45:662–666.
12. **Bieglmayer C, Scheuba C, Niederle B, Flores J, Vierhapper H.** Screening for medullary thyroid carcinoma: experience with different immunoassays for human calcitonin. *Wien Klin Wochenschr*. 2002;114:267–273.
13. **Dralle H, Damm I, Scheumann GF, et al.** Compartment-oriented microdissection of regional lymph nodes in medullary thyroid carcinoma. *Surg Today*. 1994;24:112–121.
14. **Dralle H.** Lymph node dissection and medullary thyroid carcinoma. *Br J Surg*. 2002;89:1073–1075.
15. **Dralle H.** Malignant thyroid tumors [in German]. In: Garbe C, Albers P, Beckmann MW, et al, eds. *Abbreviated Interdisciplinary Guidelines 2006. Recommendations for Diagnosis and Therapy of Malignant Diseases*. W. Munich, Germany: Zuckschwerdt Verlag; 2006.
16. **Hedinger C, Williams ED, Sobin LH.** Histological typing of thyroid tumours. In: *International Histological Classification of Tumours*. 2nd ed. Geneva: World Health Organization; 1988.
17. **DeLellis RA Lloyd RV, Heitz PU, Eng C, eds.** *World Health Orga-*

nization Classification of Tumours: Pathology and Genetics of Tumours of Endocrine Organs. Lyon, France: IARC Press; 2004.
18. **Giovanella L, Verburg FA, Imperiali M, Valabrega S, Trimboli P, Ceriani L.** Comparison of serum calcitonin and procalcitonin in detecting medullary thyroid carcinoma among patients with thyroid nodules. *Clin Chem Lab Med*. 2013;51:1477–1481.
19. **Giovanella L, Maffioli M, Suriano S, Dorizzi RM.** Elevated calcitonin and procalcitonin levels in nonmedullary benign and malignant thyroid nodules. *Clin Endocrinol (Oxf)*. 2010;72:852–853.
20. **Walter MA, Meier C, Radimerski T, et al.** Procalcitonin levels predict clinical course and progression-free survival in patients with medullary thyroid cancer. *Cancer*. 2010;116:31–40.
21. **Machens A, Hoffmann F, Sekulla C, Dralle H.** Importance of gender-specific calcitonin thresholds in screening for occult sporadic medullary thyroid cancer. *Endocr Relat Cancer*. 2009;16:1291–1298.
22. **Bihan H, Becker KL, Snider RH, et al.** Calcitonin precursor levels in human medullary thyroid carcinoma. *Thyroid*. 2003;8:819–822.
23. **Kaczka K, Mikosiński S, Fendler W, Celnik A, Pomorski L.** Calcitonin and procalcitonin in patients with medullary thyroid cancer or bacterial infection. *Adv Clin Exp Med*. 2012;21:169–178.
24. **McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics.** Reporting recommendations for tumor marker prognostic studies (REMARK). *J Natl Cancer Inst*. 2005: 97:1180–1184.
25. **Mandrekar SJ, Sargent DJ.** Clinical trial designs for predictive biomarker validation: theoretical considerations and practical challenges. *J Clin Oncol*. 2009;27:4027–4034.
26. **Bugalho MJ, Madureira D, Domingues R, Pereira T, Cortez L.** Medullary thyroid carcinoma preferentially secreting procalcitonin. *Thyroid*. 2014;24:1190-1191. 2013.0664.