Small medullary thyroid carcinoma: post-operative calcitonin rather than tumour size predicts disease persistence and progression

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Abstract

Objective: Recently, small medullary thyroid carcinomas (smallMTCs; ≤1.5 cm) are frequently diagnosed, occasionally as incidental findings in surgical specimens. Their clinical course varies. We examined tumour size as a predictor of clinical behaviour.

Design: A retrospective study.

Methods: A total of 128 smallMTC patients (35.2% males and 45% familial) were followed up for 0.9–30.9 years. According to tumour size (cm), patients were classified into four groups: group 1, 0.1–0.5 (n = 33); group 2, 0.6–0.8 (n = 33); group 3, 0.8–1.0 (n = 29) and group 4, 1.1–1.5 (n = 33).

Results: Pre- and post-operative calcitonin levels were positively associated with the tumour size (P < 0.001). Capsular and lymph node invasion were more frequent in groups 3 and 4 (P < 0.03); the stage was more advanced and the outcome was less favourable with an increasing tumour size (P < 0.001). Groups 1 and 2 patients were more frequently cured (group 1, 87.8%; group 2, 72.7%; group 3, 68.9%; and group 4, 48.5%; P = 0.002). The 10-year probability of lack of disease progression according to the tumour size differed between patients with tumour sizes of 0.1–1.0 and 1.1–1.5 cm (96.6%, 81.3%, x² = 4.03, P = 0.045 for log-rank test). Post-operative calcitonin was the only predictor significantly associated with the 10-year progression of disease. Post-operative calcitonin levels ≥4.65 pg/ml predicted disease persistence (sensitivity 93.8% and specificity 90%) and ≥14.5 pg/ml predicted disease progression (sensitivity 100%, specificity 82%, receiver operating characteristic curve analysis).

Conclusions: Tumour size may be of clinical importance only in patients with MTCs >1 cm in size. Post-operative calcitonin is a more important predictor than size for disease progression.

Introduction

Medullary thyroid carcinoma (MTC) are rare thyroid tumours of neuroendocrine origin, accounting for 2–10% of thyroid carcinomas. MTCs may present, already at the time of diagnosis, with extensive cervical lymph node involvement and occasionally with distant metastases.

SmallMTC tumours (≤1.5 cm in size) are recently diagnosed more frequently, possibly because of routine calcitonin measurement in nodular disease (1, 2, 3, 4, 5). SmallMTCs are frequently (10–15%) incidental findings in MTC surgical specimens (6, 7, 8). Their clinical significance and natural history are not clear.
In autopsy, occult MTCs were found in 0.2–0.8% of thyroid carcinomas (9). A meta-analysis of 1797 autopsies has shown that none of these tumours presented with local extension or distant metastases (10). On the other hand, data from clinical studies are conflicting. Some studies have shown no aggressive behaviour. Hamy et al. only observed lymph node involvement; thus, older studies have questioned the extent of surgical treatment needed (7, 11, 12). However, two recent studies have reported that even small tumours may present with more advanced disease (4, 13). Finally, in patients with a hereditary MTC, who frequently have small tumours due to earlier diagnosis, the clinical course and prognosis are better (13).

Prognostic factors for MTCs include tumour extent at presentation, which appears to be a significant predictor of life expectancy (14). As diagnosis was established earlier, smaller and/or less extensive tumours with a better outcome may be more frequently detected. Thus, whether tumour size could predict the clinical behaviour of MTCs would be an important question. This would be of importance not only for the prognosis of the disease but also for the surgical procedure to be followed (4, 7, 13, 15, 16). Towards this direction, there have been many efforts to establish a cut-off value of tumour size that could distinguish those patients who are at a higher risk for progressive disease.

In order to evaluate the clinical significance of small MTCs (≤1.5 cm), we examined the clinical and biochemical parameters at diagnosis and during follow-up in our cohort of MTC patients focusing on tumour size.

Subjects and methods

A total of 214 (36.0% males) patients, diagnosed with MTCs, presented to the Endocrine Unit of the Academic Department of Clinical Therapeutics, during the last 36 years. Two patients with increased calcitonin levels and nodular disease did not undergo surgery and were excluded from the analysis.

Of the patients who underwent surgery, a significant number had relatively small-sized tumours. We compared data between smaller and larger tumours using various cut-off points to identify which size could be better associated with prognosis. A tumour size of >1.5 cm was found to be associated with more severe disease, more advanced disease stage at diagnosis and less favourable outcome at follow-up. Moreover, three patients with tumour size of 1.6–2.0 cm and six patients with tumour size of >2.0 cm had distant metastases at diagnosis (stage IVC), while no patient with tumours measuring ≤1.5 cm in size had such metastasis. Of the total number of patients, 128 (60.0% of all MTCs) had tumours of ≤1.5 cm in size; these comprised the group of small MTCs; of these 95 had microcarcinomas ≤1.0 cm (micro MTCs).

Patients with small MTCs were classified into four groups according to the tumour size. Group 1 had a tumour size of 0.1–0.5 cm (n=33); group 2, 0.6–0.8 cm (n=33); group 3: 0.8–1.0 cm (n=29); and group 4, 1.1–1.5 cm (n=33). They were followed up for 0.9–30.9 years (median 4.0 years, 25.2% had follow-up ≥10 years). We focused further on the analysis mainly of small MTCs to see whether the size was of importance in this group of relatively better prognosis. We recorded the age at diagnosis and the family history of MTCs. Family history of MTCs was considered on the basis of positive RET mutation and/or positive family history of MTCs. Genetic screening for RET mutation was routinely carried out from the year 2001 onwards. It should be noted that our unit is a referral centre for MEN2 syndromes. Patients who were RET carriers, in whom histology showed C-cell hyperplasia, were not included.

This study was approved by the institutional review committee and was conducted according to the Helsinki Declaration. All patients except those lost to follow-up were informed about the aim of the study and they gave their consent.

Calcitonin screening was routinely carried out in our centre since 2001 in patients with nodular thyroid disease. Calcitonin was measured using a two-site chemiluminescent immunometric assay (Immulyte 2000, Siemens Healthcare Diagnostics Products Ltd. Llanberis, Gwynedd, UK) from 2006 to 2012 and using a chemiluminescence immunoassay (Nichol Institute Diagnostics, San Juan Capistrano, CA, USA) from 2000 to 2005. Before 2000, the ELISA–hCT Kit (Cis Bio International, IRMA, Cis-Diagnostics Gif-sur-Yvette Cedex, France) was used. As the calcitonin method used before 2006 was less sensitive, we looked at the trend of post-operative calcitonin values over time for each patient and did not observe changes potentially due to the method used. Calcitonin in patients in remission, diagnosed before 2006, was measured during the follow-up by the sensitive method, confirming the very low calcitonin levels measured earlier. Patients who were biochemically cured were re-evaluated with ultrasound imaging. Of the patients diagnosed before 2006, only ten were lost to follow-up.

The tumour size and the extent of the disease at diagnosis and during follow-up, the number of surgeries...
performed and the pre- and post-operative calcitonins were recorded. Staging at diagnosis was performed according to the American Joint Committee on Cancer (AJCC) TNM classification (17). The majority of surgeries were performed in three different collaborating surgery units. All surgeons were equally experienced in surgical procedures for thyroid cancer. Pathology was examined by the same group of two pathologists in the majority of cases. The choice of surgical procedure was made according to the preoperative calcitonin levels, the neck ultrasound findings as well as the visualisation of suspected lymph nodes during the surgical procedure as suggested previously (14, 18). Basal calcitonin and post-operative calcitonin were evaluated 3 and 6 months, and yearly after the first surgery to classify patients as having remission, stable or progressive disease. Patients with normal post-operative calcitonin levels (<1.5 pg/ml) had remission, those with measurable post-operative calcitonin levels with no new imaging lesions had stable disease and those with new or increasing size lesions had progressive disease according to the RECIST guidelines.

**Statistical analyses**

Statistical analyses was carried out using the SPSS Statistical Package (version 18). All descriptive data are expressed as mean ± S.D. for normally distributed variables, otherwise median value and intraquartile range (IQR) are shown. The χ²-test and the χ²-test for linear association (Mantel–Haenzel χ²) were used for contingency tables. For the comparison of the means, the t-test or the Mann–Whitney U rank test was used depending on the normality of distribution. ANOVA or the Kruskal–Wallis test was used as appropriate. The linear regression model was used for correlations between continuous variables (Pearson's correlation); for variables not normally distributed, Spearman's correlation was used. Binary logistic regression analysis was used for multivariate analysis.

The Kaplan–Meier product-limit method was used to estimate the probability of progression of disease 10 years (120 months) after initial diagnosis. To evaluate which factors contribute to the progression of the disease, a univariate Cox proportional hazards model was used; factors contributing to the outcome in univariate analysis at P<0.05 (due to the risk of type II error attributable to low statistical power in such an analysis) were included in the multivariate model as potential risk factors. In the final multivariate analysis, statistical significance was set at 5% (P<0.05). Receiver operating characteristic (ROC) curve analysis was used to assess the predictive value of pre- and post-operative calcitonin levels for disease progression and for recognition of a clinically relevant cut-off value. The incremental value of post-operative calcitonin over the baseline model (preoperative calcitonin) for predicting progression of the disease was evaluated comparing the areas under the curve by c-statistics. Curves were constructed by plotting sensitivity against 1-specificity. Power analysis was conducted for the primary endpoint of our study (10-year probability of disease progression according to the tumour size) using the software package, G Power 3.0.10.

**Results**

In the whole cohort, the 10-year probability of lack of progression of disease according to the tumour size was 92.6% for small MTCs and 55.4% for large MTCs (Kaplan–Meier analysis, χ²=25.1; P<0.001 for log-rank test).

In the group with small MTCs (≤1.5 cm, n=128, mean age 40.8±17.26, males 53.2% and family history 45.0%), the extent and stage of the disease at diagnosis and in the outcome did not differ according to family history (sporadic vs familial: stage I, 75.9 vs 73.4%; stage III, 18.5 vs 23.4%; and stage IV, 8.5 vs 12.7%; P=0.68; disease remission, 69.8 vs 67.6%; persistence, 17 vs 29% and progression, 13.2 vs 3.2% respectively; P=0.066). As expected, multifocality was more frequent in familial cases compared with sporadic ones (64.6 vs 14.8%; P<0.001). Preoperative calcitonin level was higher in sporadic MTCs compared with the familial MTCs (median 122 (IQR 290) vs 63.7 (170) pg/ml respectively; P=0.008). Post-operative calcitonin levels did not differ according to family history. Familial cases diagnosed after genetic screening were younger than sporadic and index cases of familial disease (25.12±14.05 vs 46.78±14.58 years; P<0.001).

Furthermore, small MTCs were divided into four subgroups according to the tumour size as mentioned above. The clinical characteristics are shown in Table 1. Capsular invasion and lymph node invasion were less frequent in groups 1 and 2 compared with groups 3 and 4 (P<0.001; Fig. 1). Patients with cervical lymph node metastases had an increased mean tumour size (lymph node negative: median 0.8 cm (IQR 0.5) vs lymph node positive: median 1.1 cm (IQR 0.5); P<0.001, Mann–Whitney U rank test). Similarly, patients with thyroid capsular invasion had a larger mean tumour size (no capsular invasion: median 0.8 cm (IQR 0.5) vs capsular invasion: median 1.0 cm (IQR 0.5); P<0.011, Mann–Whitney U rank test).
The stage of disease at diagnosis was advanced with an increasing tumour size ($x^2$, $P=0.001$; Table 1). Five patients were diagnosed with disease stage IV: two sporadic MTC patients belonging to group 1 (primary tumour size of 0.3 and 0.4 cm) were diagnosed because of palpable metastatic cervical lymph nodes, and three further patients belonging to group 4 were diagnosed after calcitonin measurement for nodular goitre and lymphadenopathy. No distant metastasis at diagnosis was observed in any of the patients belonging to the four groups.

Mean pre- and post-operative calcitonin levels increased according to the size in the four subgroups ($P<0.001$ and $P=0.001$, Kruskal–Wallis test; Table 2). Undetectable post-operative calcitonin level ($\leq 1.5$ pg/ml) after the first surgery was less frequently observed in group 4 (group 1, 79.3%; group 2, 62.5%; group 3, 61.5% and group 4, 46.9%; $P=0.013$). In patients who underwent only total or subtotal thyroidectomy, no differences in the undetectable calcitonin rate after the first surgery were observed (86.4% of group 1, 61.1% of group 2, 76.9% of group 3 and 62.5% of group 4; $P=0.2$). Only two patients with undetectable post-operative calcitonin levels had recurrence of disease; the disease in those patients remained stable at follow-up. Another five patients had stable disease for at least 2 years but showed disease progression afterwards. In multivariate logistic regression analysis, when the extent of first surgery, tumour size, lymph node positivity, capsular and soft tissue invasion as well as preoperative calcitonin were taken into account with capsular invasion and preoperative calcitonin were predictors of undetectable post-operative calcitonin (OR: 0.161, 95% CI: 0.04–0.67; $P=0.012$ and OR: 1.003, 95% CI: 1.00–1.08; $P=0.033$).

The outcome of the disease was more favourable with a decreasing tumour size in the four subgroups ($P=0.002$; Table 1). In the group with remission, the mean size was smaller (median: 0.8 cm, IQR: 0.5) compared with the

Table 1 Baseline demographic characteristics, mode of diagnosis, disease stage and surgical treatment according to the tumour size in patients with small MTCs ($\leq 1.5$ cm) divided into four subgroups.

<table>
<thead>
<tr>
<th>Largest tumour diameter (cm)</th>
<th>Group 1: 0.1–0.5, $n=33$</th>
<th>Group 2: 0.6–0.8, $n=33$</th>
<th>Group 3: 0.9–1.0, $n=29$</th>
<th>Group 4: 1.1–1.5, $n=33$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years $\pm$ s.d.)</td>
<td>32.83 $\pm$ 17.1</td>
<td>45.84 $\pm$ 14.1</td>
<td>45.08 $\pm$ 18.5</td>
<td>41.88 $\pm$ 16.5</td>
<td>0.011</td>
</tr>
<tr>
<td>Sex: males, $n$ (%)</td>
<td>13 (39.4)</td>
<td>10 (30.3)</td>
<td>12 (41.4)</td>
<td>10 (30.3)</td>
<td>0.6</td>
</tr>
<tr>
<td>Family history: sporadic, $n$ (%)</td>
<td>13 (39.4)</td>
<td>10 (30.3)</td>
<td>12 (41.4)</td>
<td>15 (45.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>TNM stage, $n$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>29 (87.9)</td>
<td>29 (87.9)</td>
<td>21 (72.4)</td>
<td>17 (51.5)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2 (6.1)</td>
<td>4 (12.1)</td>
<td>8 (27.6)</td>
<td>13 (39.4)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>IVA</td>
<td>1 (3.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (6.1)</td>
<td></td>
</tr>
<tr>
<td>IVB</td>
<td>1 (3.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (3.0)</td>
<td></td>
</tr>
<tr>
<td>IVC</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Years of follow-up ($\pm$ s.d.)</td>
<td>5.5 $\pm$ 5.5</td>
<td>4.60 $\pm$ 5.1</td>
<td>4.9 $\pm$ 4.92</td>
<td>7.65 $\pm$ 6.8</td>
<td>0.069</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>2.5 (8.3)</td>
<td>3 (4)</td>
<td>3 (3.8)</td>
<td>5 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Mode of diagnosis, $n$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcitonin measurement</td>
<td>13 (39.4)</td>
<td>21 (63.6)</td>
<td>17 (58.8)</td>
<td>21 (63.7)</td>
<td></td>
</tr>
<tr>
<td>Surgery for nodular goitre</td>
<td>4 (12.2)</td>
<td>3 (9.1)</td>
<td>3 (10.3)</td>
<td>3 (9.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>FNA</td>
<td>1 (3.0)</td>
<td>3 (9.1)</td>
<td>3 (10.3)</td>
<td>2 (6.0)</td>
<td></td>
</tr>
<tr>
<td>Genetic screening</td>
<td>15 (45.4)</td>
<td>6 (18.2)</td>
<td>6 (20.6)</td>
<td>7 (21.2)</td>
<td></td>
</tr>
<tr>
<td>Type of first surgery, $n$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, near total thyroidectomy</td>
<td>26 (78.8)</td>
<td>19 (57.6)</td>
<td>16 (55.2)</td>
<td>8 (24.3)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Total thyroidectomy and central lymph node dissection</td>
<td>7 (21.2)</td>
<td>14 (42.4)</td>
<td>13 (44.8)</td>
<td>25 (75.7)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Number of surgeries, $n$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$\geq 2$</td>
<td>31 (94.0)</td>
<td>28 (84.8)</td>
<td>25 (86.2)</td>
<td>22 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Outcome, $n$ (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Remission</td>
<td>29 (88)</td>
<td>25 (75.7)</td>
<td>20 (69)</td>
<td>17 (51.5)</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>2 (6)</td>
<td>7 (21.2)</td>
<td>9 (31)</td>
<td>10 (30.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>2 (6)</td>
<td>1 (3.1)</td>
<td>0 (0)</td>
<td>6 (18.2)</td>
<td></td>
</tr>
</tbody>
</table>

FNA, fine needle aspiration
group with stable (1.0 cm, IQR: 0.5) or progressive (1.2 cm, IQR: 0.8) disease (Kruskal–Wallis test, \( P = 0.007 \)). Two group 4 patients had distant metastases at follow-up; the two group 1 patients with stage IV disease at diagnosis presented with rapidly progressive disease and distant metastases during follow-up.

The 10-year probability of lack of progression of disease according to the tumour size did not differ significantly among the four groups in the overall analysis (for tumour size 0.1–0.5, 93.3%; for 0.6–0.8, 96.9%; for 0.9–1.0, 100% and for 1.1–1.5, 81.3%; Kaplan–Meier analysis, \( x^2 = 4.75, P = 0.19 \) for log-rank test). When the two group 1 patients with microscopic but aggressive tumours were excluded from the analysis, there was a significant difference (for tumour size of 0.1–0.5 cm, lack of progression of disease: 100%, \( x^2 = 8.37, P = 0.039 \) for log-rank test). The 10-year probability of lack of progression of disease differed significantly when patients with tumour sizes of 0.1–1.0 and 1.1–1.5 cm were compared (96.6 vs 81.3%, Kaplan–Meier analysis, \( x^2 = 4.03, P = 0.045 \) for log-rank test; Fig. 2); similar results were obtained after the exclusion of the two group 1 patients (98.8 vs 81.3% respectively, \( x^2 = 7.86, P = 0.005 \)). In Cox proportional hazard analysis where tumour size, surgery extent, disease stage at diagnosis, and pre- and post-operative calcitonins were taken into account, the only predictor of 10-year disease progression was post-operative calcitonin (HR: 1.011, 95% CI: 1.005–1.016, \( P < 0.001 \)). No difference in the results was found when either the method of calcitonin measurement (used in our laboratory before 2000, between 2001 and 2005 and after 2005) or the post-operative calcitonin doubling time was also taken into account. All nine patients with disease progression had post-operative calcitonin levels \( \geq 14.5 \) pg/ml yielding a sensitivity of 100% and specificity of 82% by ROC analysis for this cut-off to predict disease progression (Fig. 3). Post-operative calcitonin levels incrementally predicted over preoperative calcitonin the disease progression (\( P = 0.001; \) Fig. 4). In these patients, post-operative calcitonin doubling time was 1.84 ± 1.1 years (range 0.6–4 years); patients with stable disease had also stable calcitonin levels during follow-up.

Patients belonging to groups 1 and 2 were more frequently cured compared with groups 3 and 4 (group 1, 87.8%; group 2, 72.7%; group 3, 68.9% and group 4, 48.5%; \( P = 0.002 \)). However, in multivariate logistic regression analysis, when the extent of first surgery, tumour size, disease stage at diagnosis and pre- and post-operative calcitonins were taken into account, the only predictor of cure was post-operative calcitonin (OR: 1.126, 95% CI: 1.049–1.207; \( P = 0.001 \)). Moreover, post-operative calcitonin level at \( \geq 4.65 \) pg/ml predicted persistence of disease with 93.8% sensitivity and 90% specificity by ROC analysis (Fig. 3).

<table>
<thead>
<tr>
<th>Largest tumour diameter (cm)</th>
<th>Group 1: 0.1–0.5, ( n = 30 )</th>
<th>Group 2: 0.6–0.8, ( n = 31 )</th>
<th>Group 3: 0.9–1.0, ( n = 26 )</th>
<th>Group 4: 1.1–1.5, ( n = 32 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative calcitonin levels (pg/ml), median (IQR)</td>
<td>33.3 (15.1)</td>
<td>82.5 (111.7)</td>
<td>140.5 (339.7)</td>
<td>189 (400.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Post-operative calcitonin levels (g/l pg per ml), median (IQR)</td>
<td>1.0 (4.1)</td>
<td>1.2 (8.57)</td>
<td>1.9 (10.4)</td>
<td>3.4 (63.7)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Further variables that might influence cure rates were examined. Thus, 82.6% of patients without lymph node invasion at diagnosis were cured compared with only 28.6% of those with positive lymph nodes ($P < 0.001$). Of those without capsular invasion, 81.8% were cured compared with 28.6% with capsular invasion ($P < 0.001$). Similarly, of those without soft tissue invasion, 73.6% were cured compared with 33.3% with tissue invasion ($P = 0.012$). In patients who had no lymph node invasion in the histological specimens, including those patients who did not undergo lymph node dissection, no differences in post-operative calcitonin levels were found according to the type of surgery (only thyroidectomy: median 1.05 pg/ml (IQR 4.5) vs thyroidectomy with lymph node dissection: median 1.7 (IQR 5.2)).

Post hoc analysis indicated that the recruited sample size of 128 subjects provided 99% power to detect a significant association between the tumour size (recoded as highest vs lower quartiles of distribution) and disease progression at the 5% level. Moreover, the study design provided over 95% power to detect a significant inverse association of the tumour size with cure of the disease assuming a sample size of 30 subjects per group and an observed mean decrease of 3% in cure probabilities across ascending categories of tumour size. Finally, for power calculations implicating distribution of calcitonin across categories of tumour size, a medium effect size index $w = 0.422$ according to Cohen’s conventions was calculated and observed power exceeded 90% assuming a homogeneous variance within tumour size categories.

A separate analysis was performed in the group with microcarcinomas (tumour size $\leq 1.0$ cm, 45% of all MTC cases). Various size cut-off values were used in this group to compare the clinical parameters. The size cut-off for less
advanced disease was found to be 0.5 cm: patients with a tumour size of $\leq$0.5 cm had less advanced stage at diagnosis compared with those with a tumour size of 0.6–1.0 cm (stages I and II, 87.9 vs 81.8%; stage III, 6.1 vs 18.2% and stage IV, 6.1 vs 0%; $P=0.048$). Preoperative calcitonin levels were lower in patients with a tumour size of $\leq$0.5 cm compared with those with a tumour size of 0.6–1.0 cm (median: 32.4 pg/ml (IQR 35.2) vs 115.5 pg/ml (IQR 210.5); $P<0.001$). Similarly, post-operative calcitonin levels were lower (median: 0.5 pg/ml (IQR 1.9) vs 1.8 pg/ml (IQR 11.2); $P=0.003$). Of the total number of patients, 67.8% with microcarcinomas had undetectable post-operative calcitonin levels ($\leq$1.5 pg/ml). Of those with negative lymph nodes at diagnosis, 76.8% had undetectable post-operative calcitonin compared with only 28.6% with positive lymph nodes ($P=0.001$). Remission was more frequent in patients with a tumour size of 0.1–0.5 cm (87.8 vs 75.7%); however, progression was slightly higher (6.1 vs 3.0%) as the two patients with aggressive tumours happened to belong to this subgroup ($P=0.054$). However, when these patients were excluded from the analysis, the outcome was more favourable in patients with a tumour size of $\leq$0.5 cm (remission, 93.9 vs 71%; stable disease, 6.1 vs 27.4% and progression, 0.0 vs 1.6%; $P=0.028$).

Discussion

The entity of microMTCs has recently been a topic of discussion and controversies concerning its prognosis (10, 13). In this retrospective study of MTC patients, we aimed to investigate whether tumour size could be of prognostic value for the clinical behaviour of MTCs, especially of smallMTCs ($\leq$1.5 cm); the aggressiveness of larger tumours has previously been shown (1, 13, 16, 19, 20). We divided smallMTCs into four size subgroups roughly representing quartiles and compared the clinical features between subgroups.

Within smallMTCs, more advanced stage at diagnosis was observed with an increasing tumour size, confirming previous findings (13, 14, 15). Pre- and post-operative calcitonin was also associated with the tumour size (14, 21). Detectable post-operative calcitonin was more frequent with an increasing tumour size, agreeing with previous reports (15). Machens et al. (13) reported that 16–24% microMTC patients had not attained normal post-operative calcitonin, while Beressi et al. (19) reported that increased post-operative calcitonin level was found in 29% of patients with microMTCs. In an older study (22) in microMTC patients who underwent total thyroidectomy with lymph node dissection, ‘normalisation’ of post-operative calcitonin was observed in all. It is likely that the criterion of normalisation is currently different from the one used in older studies.

The outcome of the disease was more favourable with a decreasing tumour size. Only two patients with a tumour size of 0.3–0.4 cm had distant metastases during follow-up, one of whom had rapidly progressive disease and died 3 years after diagnosis. The 10-year probability of lack of progression of disease according to the tumour size differed significantly when patients with tumour sizes of 0.1–1.0 and 1.1–1.5 cm were compared (96.6 vs 81.3%). In older series, only survival rates were reported and appeared to indicate more rapidly progressing disease (19, 23). Thus, most of our patients with detectable calcitonin post-operatively had stable disease, with low calcitonin and negative imaging.

Furthermore, we examined various factors that could predict disease progression. Interestingly, in Cox proportional hazard analysis, post-operative calcitonin was the only significant predictor for the 10-year progression of disease while size was not a significant predictor. Moreover, ROC analysis revealed that post-operative calcitonin levels $\geq$14.5 pg/ml predicted progression with 100% sensitivity and 82% specificity. Concerning the cure rates, again post-operative calcitonin levels $\geq$4.65 pg/ml predicted persistence with 93.8% sensitivity and 90% specificity, suggesting that the predictive value of post-operative calcitonin was additive to that of preoperative calcitonin for disease progression. This finding has not
been previously reported; these cut-off values of postoperative calcitonin could indicate the need for increased awareness. Moreover, this finding may emphasise the need for a better surgical management during the first therapeutic intervention in MTC patients irrespective of size (17). Other investigators have reported that either tumour size and stage or age (19, 23) is the best predictor of survival and cure. In another study, preoperative calcitonin and stage were predictors of remission (21). Finally, it should be noted that the results of our study showed that the prognosis is based on the post-operative calcitonin levels and not the TNM stage, which is a classical preoperative predictor. Therefore it appears that, similar to the case of follicular cell-derived thyroid cancer, in MTCs as well, the prognosis is an evolving concept that should be re-evaluated at various steps of the treatment and follow-up.

As the majority of the relevant published series study microMTCs (≤1 cm), we performed a separate analysis in patients with a tumour size of ≤1.0 cm using various cut-off values. Patients with a tumour size of ≤0.5 cm had less advanced stage at diagnosis and a tendency for more favourable outcome of disease; furthermore, when the two patients with microscopic aggressive tumours were excluded, the outcome was more favourable in patients with a tumour size of ≤0.5 cm. This threshold agrees with a recent report suggesting more aggressive behaviour in tumours measuring >0.5 cm in size (13) and cure rates of 80% (13). In other studies, cure rates of 71–100% were reported (11, 19, 22, 24). The variance in cure rates may be due to differences in the chronological periods and the ratio of familial disease. Death in microMTC patients has been reported in older studies where the 10-year survival was 96% for localised tumours and 87% for regional tumours (4, 19, 24). In microMTC patients with positive lymph nodes at diagnosis, undetectable post-operative calcitonin was found in 28.6% compared with 76.8% in those without positive lymph nodes at diagnosis, roughly agreeing with the findings of Scollo et al. (15) (32 vs 95%). However, there are controversies in the literature concerning the need for systemic lymph node dissection in lesions ≤0.5 cm in size (4, 7, 11, 12, 13). It is possible that the definition of lymph node dissection may have varied in these studies (25). Thus, we recommend that a tumour size of ≥0.5 cm might be a threshold for central lymph node dissection not only for the familial cases but also for the sporadic ones, as also suggested by other authors (13, 17).

MicroMTCs may rarely show an aggressive behaviour, as in our two patients who were diagnosed because of the lymph node enlargement and where the MTCs were not incidental findings. The genetic background and specific molecular events might be responsible for such differences (26). Nevertheless, early diagnosis and the appropriate surgical management can improve the outcome (4, 7, 11, 12, 13, 16, 27). It should be recognised that the majority of small-sized MTCs appear to be innocent not affecting the overall survival (6, 16, 28). Molecular markers may help identify those tumours with an increased risk of aggressiveness in the future. From our data, we cannot suggest any new criteria for identifying the very rare ‘really high risk’ patients with MTCs at an early stage, as we only observed one death.

There are several limitations in our study. The sample is rather small; however, power analysis showed that this was sufficient. Furthermore, some of our patients were diagnosed before 1990, when less sensitive calcitonin assays, imaging and less extensive lymph node dissections were performed. It is likely that our results are influenced by the fact that more small tumours have been detected recently. With regard to our results, some issues need to be considered. As an MTC is a tumour with usually slow progression, we were able to re-evaluate the majority of our patients with the more sensitive calcitonin assays. In addition, as lymph node dissection was not performed in all patients, we retrospectively examined those patients without lymph node dissection taking into account both the pre- and especially the post-surgical ultrasound, and we were able to confirm that the stage at diagnosis was correct. Furthermore, when analysis was performed in the subgroup of patients with no lymph node invasion, either with or without lymph node dissection, post-operative calcitonin levels did not differ, thus excluding the possibility that some patients were misclassified in terms of staging. Finally, another limitation of our study is the short period of follow-up with a median of 4 years. Considering the longevity of MTC patients and the fact that recurrence of the disease may occur later than the first 3 years, it cannot be excluded that for some of our patients with microcarcinomas, the longer follow-up may change their classification.

**Conclusions**

Tumour size may be of clinical importance for the progression of disease only in patients with MTCs >1 cm. Other histological characteristics such as lymph node invasion and extrathyroidal extension may be of value in determining those patients who are at risk for persistence or even progressive disease. When the group of smallMTCs (≤1.5 cm) is considered, again the probability
of 10-year disease progression slightly increases in those with a tumour size of >1.0 cm. This study confirmed that post-operative calcitonin is a more important predictor than size for disease progression. Thresholds of 4.6 and 14.5 pg/ml may predict the risk for disease persistence and progression respectively with high sensitivity and specificity. In the subgroup of microMTCs (≤1.0 cm), the stage appears to be less advanced in tumours of ≤0.5 cm in size, while the outcome tends to be more favourable in those patients with a tumour size of 0.1–0.5 cm although, rarely, microscopic tumours may present with a more aggressive disease.

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

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Author contribution statement
K Saltiki participated in the design of the study, drafted the manuscript and performed the statistical analysis. G Rentziou, K Stamatelopoulos, G Georgiopoulou, C Stavrianos, and E Lamberinoudaki collected the data and participated in the statistical analysis. All authors read and approved the final manuscript. M Alevizaki conceived the study and participated in its design and coordination.

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