Fine-needle aspiration cytology for medullary thyroid carcinoma: a single institutional experience in Japan

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Abstract. Many cytological studies on medullary thyroid carcinoma (MTC) have been reported; however, such studies in large series of patients with MTC have not been performed. We investigated MTC at a single institution in Japan using fine-needle aspiration cytology (FNAC), and aimed to establish a preoperative diagnostic algorithm for MTC. FNAC was performed in 119 of 149 patients with MTC (79.9%) who ultimately underwent surgical resection. Moreover, 22 of 56 hereditary MTC (39.3%) were diagnosed preoperatively without FNAC by their high serum calcitonin levels or increased response to calcium stimulation (11 cases each), as well as RET mutation analysis. On FNAC, 76.5% of nodules were categorized as ‘malignancy’ or ‘suspicious for malignancy’. The sensitivity and specificity of calcitonin measurement in aspiration needle wash-out fluid and in immunocytochemical staining for calcitonin were 96.3% and 92.3% respectively. We proposed an algorithm for preoperative diagnosis of MTC utilizing FNAC: When thyroid nodules are highly suspicious for MTC by their clinical and ultrasonographic features, serum calcitonin measurement with or without a calcium stimulation test is required. Furthermore, FNAC should be performed for patients who do not have those findings. When there is a possibility of MTC at the time of FNAC, calcitonin measurement using needle wash-out fluid is a reliable diagnostic tool. When MTC is suspected on cytological examination, immunocytochemical staining for calcitonin is useful for confirming MTC diagnosis.

Key words: Medullary thyroid carcinoma, Calcitonin, Needle wash-out fluid, Immunocytochemical staining, Fine-needle aspiration cytology

Materials and Methods

The study protocol was reviewed and approved by the Institutional Review Board of Kuma Hospital.

We reviewed 11,940 patients with thyroid carcinoma who underwent resection at Kuma Hospital between January 2007 and December 2016. The diagnosis of MTC necessarily involved histological and immunohistochemical examination using antibodies against calcitonin (rabbit polyclonal, ready to use,
Dako, Denmark, Glostrup) and carcinoembryonic antigen (CEA; COL-1 clone, ready to use, Nichirei, Tokyo, Japan). Among these patients, 149 (1.2%) had MTC; 93 (62.4%) and 56 (37.6%) were sporadic and hereditary forms, respectively. Sporadic MTC occurred in 60 women and 33 men with a median age of 61 years (range, 26–81 years). Hereditary MTC afflicted 36 women and 20 men with a median age of 36.5 years (range, 8–72 years).

During the same period, 74,129 patients underwent FNAC for 101,104 thyroid nodules. Among them, 197 nodules (0.2%) were suspicious for MTC or could not be ruled out as MTC on cytological examination. FNAC was performed by using a 22-gauge needle under ultrasound guidance. Cytological slides were prepared by expressing the aspirated materials from the needle and sandwiching them between glass slides; they were immediately fixed with Cytorop (Alfresa Pharma Co., Osaka, Japan). The samples were then stained using the Papanicolaou method. We retrospectively examined preoperative FNAC results in 149 nodules in which MTC was histologically confirmed and in another 197 nodules in which MTC was cytologically suspected. Calcitonin measurements in aspiration needle wash-out fluid were performed in 515 nodules. After smearing, the remaining aspirates in the syringe and needle were rinsed with 0.5 mL normal saline and submitted for calcitonin measurement. Of 515 nodules, 201 (39.0%) were subsequently resected. Immunocytochemical examination using anti-calcitonin antibody was performed in 33 of 197 nodules (16.8%) that were cytologically suspected to be MTC; in 21 of them (63.6%), liquid-based cytology specimens were used. Subsequently, 27 of these nodules (81.8%) underwent surgical resection.

FNAC reports were categorized as nondiagnostic or unsatisfactory (ND/UNS), benign, atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS), follicular neoplasm or suspicious for a follicular neoplasm (FN/SFN), suspicious for malignancy (SFM), and malignant based on the criteria of “the Bethesda system for reporting thyroid cytopathology.” Clinical data were obtained from medical records of Kuma Hospital.

Results

FNAC was performed in 119 of 149 patients (79.9%) with MTC. We did not perform FNAC in 8 patients (8.6%) with sporadic MTC; 3 of these patients had undergone FNAC at another hospital while MTCs in the remaining 5 were discovered incidentally in thyroids resected because of PTC (4 cases) and Graves' disease (1 case). Preoperative diagnoses of 22 hereditary MTCs (39.3%) without FNAC were established by the high serum calcitonin level (11 patients) or the increased response to calcium stimulation (11 patients).

FNAC were performed in 136 MTC nodules in 119 patients; the results are shown in Table 1. Four nodules were ND/UNS (2.9% of aspirated nodules); aspirated cells could not be clearly observed in 3 of them because of bloody materials. In 8 nodules reported as benign (5.9%), aspirated materials were obtained from normal thyroid tissue surrounding the MTC nodules; 3 of them were suspected to be adenomatous goiter. Twenty nodules (14.7%) were AUS/FLUS, 11 of which revealed few MTC cells that were insufficient to make a definite diagnosis of MTC. The remaining 9 nodules required the ruling out of other lesions including follicular neoplasm or adenomatous goiter. There were no reported nodules of FN/SFN. Malignancy and SFM were found in 76.5% of the nodules; MTC was suggested for all except 1 nodule in which poorly differentiated carcinoma was also listed as a differential diagnosis. As for diagnostic accuracy, no significant difference between sporadic and hereditary MTCs was noted.

Of 136 MTC nodules, 18 (13.2%) underwent re-aspiration within 6 months (Table 2). The incidences of re-aspiration in sporadic and hereditary MTC nodules were 17.8% and 4.3%, respectively. Of 18 nodules, 11 (61.1%) were diagnosed as MTC. All 6 nodules that were reported as SFM or malignancy on initial aspiration and that underwent re-aspiration were sporadic MTCs.

Of 197 nodules that were suspected MTCs or for which MTC was not ruled out on thyroid FNAC, 153 (77.7%) were surgically resected at our hospital; 117 of these nodules (76.5%) were diagnosed as MTCs. Another diagnosis included PTCs (14 nodules), follicular neoplasm (8 nodules including 1 oxyphilic cell variant nodule), nodular goiter (6 nodules), poorly differentiated carcinoma (4 nodules), columnar cell carcinoma (1 nodule), carcinoma showing thymus-like differentiation (1 nodule), lymphoma (1 nodule), and Graves' disease (1 nodule).

Of 201 nodules for which calcitonin was measured in the aspiration needle wash-out fluid and that underwent subsequent resection, 107 (53.2%) were MTCs.
and 94 (46.8%) were other lesions (Table 3), including PTC (46), adenomatous nodules (22), follicular neoplasms (14), chronic thyroiditis (4), poorly differentiated carcinomas (3), Graves’ disease (1), hyalinizing trabecular tumor (1), carcinoma showing thymus-like differentiation (1), columnar cell carcinoma (1), and well-differentiated neoplasm of uncertain malignant potential (1). The calcitonin levels ranged from 14 pg/mL to 6,600,000 pg/mL in the MTC nodules. One-hundred three MTC nodules (96.3%) had calcitonin levels >100 pg/mL; in 3 of the 4 nodules with <100 pg/mL, cytological data indicated ND/UNS. Among the 94 non-MTC nodules, calcitonin levels ranged from <10 to 1,800 pg/mL; 92 nodules (97.9%) showed less than 100 pg/mL. In 2 nodules with >100 pg/mL calcitonin, the immunohistochemical examination revealed the presence of C-cell hyperplasia near the nodules. When 100 pg/mL was set as a cut-off value arbitrarily, the sensitivity, specificity, positive predictive value, and negative predictive value of the calcitonin measurement test in the aspiration needle wash-out fluid were 96.3%, 97.9%, 98.1%, and 95.8%, respectively.

Twenty-seven nodules with preoperative immunocytochemical examination using anti-calcitonin antibody included MTC (13), follicular neoplasm (10), PTC (2), and poorly differentiated carcinoma (2) (Table 4). Twelve MTC nodules were positive for calcitonin; the remaining nodule was negative because of the absence of MTC cells on the preparation. All non-MTC nodules were non-reactive. The sensitivity, specificity, positive predictive value, and negative predictive value of the immunostaining method were 92.3%, 100%, 100%, and 93.3%, respectively.

**Discussion**

In general, thyroid nodules discovered by ultrasonography or palpation undergo FNAC for diagnosis...
Suzuki et al.

However, in our series, approximately 20% of patients with MTC underwent surgical resection without preoperative FNAC; most of these patients had hereditary MTC. Preoperative diagnoses of MTC without FNAC were confirmed by high serum calcitonin levels or increased response to calcium stimulation. FNAC may not always be necessary for diagnosing MTC in patients with thyroid nodules. Pereira et al. reported that the sensitivity of the serum calcitonin level for the diagnosis of MTC was much higher (98.7%) than that of FNAC (51.3%) [12]. In patients with a family history of MTC or RET mutation, cytological evaluation of the thyroid nodules was not involved in the indication for thyroidectomy. High serum calcitonin levels or increased response to calcium stimulation are reasons to perform total thyroidectomy for the same reasons that prophylactic thyroidectomy are performed in RET gene carriers [3,13].

The diagnostic accuracy of MTC on FNAC ranges from 50.0% to 82.4% [4-7]. According to a meta-analysis by Trimboli et al., the detection rate of FNAC in patients with MTC ranged from 12.5% to 88.2%, with a pooled estimate of 56.4% [14]. In our study, the detection and diagnosis of MTC were achieved in 76.5% and 70.6% of cases, respectively. The causes of missed or overlooked diagnoses include the low incidence of MTC, a variety of cellular morphologies, nontypical cell shapes, and low cellularity [4-5]. Forrest et al. [15] described difficulties in differential diagnoses between MTC and follicular carcinoma, anaplastic carcinoma or PTC, and hyperplastic nodules. Owing to its many possible microscopic patterns, MTC is known as a “great mimicker” [2] because papillae can mimic PTC, microfollicles can mimic follicular neoplasm, a dispersed cell pattern can mimic lymphoma or plasmacytoma, and spindle or bizarre cells can mimic sarcoma or anaplastic carcinoma. Microfollicles containing amyloid resemble dense colloid [2]. Because both MTCs and PTCs often exhibit intranuclear cytoplasmic inclusions, their differential diagnoses may be challenging [2]. In our case, the following lesions were misdiagnosed or could not be differentiated (in order of frequency): PTC, follicular neoplasm, nodular goiter, and poorly differentiated carcinoma. The tumor most commonly confused with MTC is Hürthle cell neoplasm [16], although we experienced only 1 such case. On diagnostic accuracy, no significant differences between sporadic and hereditary MTCs were noted in our patients; therefore, clinical information that was suggestive of MTC did not improve the diagnostic accuracy of this disease.

According to the Bethesda system [16] for nodules with ND/UNS or AUS/FLUS cytology, repeat FNAC and/or molecular testing are recommended. In our patients, the incidences of re-aspiration in sporadic and hereditary MTC nodules with ND/UNS or AUS/FLUS cytology were 52.9% and 14.3%, respectively. As stated above, re-examination with FNAC is not necessary for patients in whom MTC is strongly suspected; high serum calcitonin levels are sufficient reason to perform surgical resection. As such, the expected role of FNAC is not to make a definite diagnosis of MTC but to reveal its possibility. However, serum calcitonin may also be increased owing to other conditions such as renal failure, neuroendocrine tumors, hypergastrinemia, hypercalcemia, pseudohypoparathyroidism, and even increased age [10,17-19]. Additionally, it is worth noting that serum calcitonin levels may not be elevated in patients with small-sized MTCs [9,20]. When FNAC is performed in cases where MTC is suspected, calcitonin measurement using needle wash-out fluid is useful to confirm the diagnosis of MTC [6,7,9,11,12,21]. The procedure may also be required in cases where serum CEA is elevated and the primary cancer is unknown [3]. The specificity and sensitivity of wash-out fluid examination are higher than those of FNAC [6,7,11]. In our study, the sensitivity was higher (96.3%) than that of FNAC (76.5%); however, false positives may occur owing to peripheral blood contamination in patients with extremely high serum calcitonin [11]. We demonstrated that C-cell hyperplasia can also reveal elevated calcitonin levels; therefore,

Table 3 The result of calcitonin measurement in the aspiration needle wash-out fluid

<table>
<thead>
<tr>
<th></th>
<th>&lt;100pg/mL</th>
<th>≥100pg/mL</th>
<th>range (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTC</td>
<td>4 (3.7%)</td>
<td>103 (96.3%)</td>
<td>14-6600000</td>
</tr>
<tr>
<td>non-MTC</td>
<td>92 (97.9%)</td>
<td>2 (2.1%)</td>
<td>&lt;10-1800</td>
</tr>
</tbody>
</table>

MTC, medullary thyroid carcinoma.

Table 4 The result of preoperative immunocytochemical examination using anti-calcitonin antibody

<table>
<thead>
<tr>
<th></th>
<th>positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTC</td>
<td>12 (92.3%)</td>
<td>1 (7.7%)</td>
</tr>
<tr>
<td>non-MTC</td>
<td>0 (0%)</td>
<td>14 (100%)</td>
</tr>
</tbody>
</table>

MTC, medullary thyroid carcinoma.
the integration of FNAC and calcitonin measurement is preferred [7].

Cytological diagnosis of MTC may be suspected but is not definitive due to poor cellularity or unusual cytomorphologic presentations. In such cases, immunocytochemical staining using anti-calcitonin antibody is adequate for diagnosing MTC if sufficient material is available [2,16,22,23]. Given that the sensitivity, specificity, positive predictive value, and negative predictive value in our study were 92.3%, 100%, 100%, and 93.3%, respectively, we recommend immunostaining for calcitonin for the definite diagnosis of MTC. However, this may be not necessary in cases of markedly elevated serum calcitonin levels.

In light of our data, and taking into account previously published studies, we propose an algorithm for the preoperative diagnosis of MTC with a focus on FNAC. This algorithm is shown in Fig. 1. When thyroid nodules are highly suspected to be MTC in the clinical setting, serum calcitonin measurements with or without calcium stimulation test are required; in such cases, FNAC is not the primary diagnostic tool. FNAC should be performed for patients whose calcitonin tests are negative. When there is a possibility of MTC at the time of FNAC, calcitonin measurement using needle wash-out fluid is a reliable tool. When MTC is suspected on cytological examination, immunocytochemical staining for calcitonin is important for making a diagnosis of MTC.

**Disclosure**

None of the authors have any potential conflicts of interest associated with this research.

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**Fig. 1** Algorithm for the diagnosis of medullary thyroid carcinoma with a focus on fine-needle aspiration cytology. MTC, medullary thyroid carcinoma; FNAC, fine-needle aspiration cytology; CT, calcitonin; CEA, carcinoembryonic antigen; ICC, Immunocytochemistry.

References


