Cytology Versus Calcitonin Assay in Fine-needle Aspiration Biopsy Wash-out Fluid (FNAB-CT) in Diagnosis of Medullary Thyroid Microcarcinoma.

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Research Article

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Abstract

**Purpose** Widespread use of sensitive ultrasound examination led to an increasing detection of medullary thyroid microcarcinoma (micro-MTC). This prospective study evaluated the diagnostic accuracy of Fine-needle Aspiration Biopsy Cytology (FNAB-C) and calcitonin assay in Fine-needle Aspiration Biopsy wash-out fluid (FNAB-CT) in thyroid nodules less than 1cm with elevated serum calcitonin.

**Methods** 87 thyroid nodules from 60 patients with elevated serum calcitonin (>10pg/ml) were included and 51 were thyroid nodules less than 1cm. FNAB-CT and FNAB-C was performed to distinguish MTC lesions before surgery, histopathologic diagnoses served as main reference standards.

**Results** FNAB-CT had a greater performance over FNAB-C for preoperative diagnosis of MTC (diagnostic accuracy: 98.85% vs 61.90%, sensitivity: 98.55% vs 55.07%, specificity: 100% vs 97.44%), especially for micro-MTC: FNAB-C established a sensitivity and diagnostic accuracy of 48.78% and 58% respectively, while FNAB-CT reached 97.56% sensitivity and 98.04% diagnostic accuracy.

**Conclusions** FNAB-CT demonstrated high diagnostic accuracy in diagnosing micro-MTC. Patients with micro thyroid nodules and elevated sCT level should perform FNAB-CT to exclude the diagnosis of MTC lesions.

Introduction

Medullary thyroid carcinoma (MTC) is a neuroendocrine malignancy originated from the calcitonin (CT)-secreting parafollicular C cells, which accounts for ~2% of all malignant thyroid neoplasms and 13.4% of the total deaths attributable to thyroid cancer [1]. According to the greatest dimension in histopathology, MTC can be classified into micro-MTC (≤10 mm) and macro-MTC (>10 mm). The routine use of sensitive ultrasound enabled the early detection of micro thyroid carcinoma and retrospective analyses suggested an increasing trend in the proportion of micro-MTC in recent decades [2-6]. Comparing with its larger counterpart, micro-MTC has a similar aggressive behavior (including extrathyroid extension, regional and distant metastases) but a relatively atypical diagnostic feature [2, 3, 7-10].

Serum calcitonin(sCT) level generally rises early in MTC patients and parallels with tumor progression. Setting a recognized cutoff as 10pg/ml, it has a sensitivity of nearly 100% in detecting MTC [11-13]. Nevertheless, as tumor size significantly correlates with preoperative sCT levels, patients with micro-MTC usually have an “indeterminate” elevated basal sCT (10-100pg/ml), making it difficult to distinguish early stage MTC from other physiological and pathological conditions [8, 14, 15]. Stimulation tests had been proposed to confirm MTC suspicious cases, especially for patients with “indeterminate” sCT [11, 12, 16]. Whereas, one recent study found no superiority of the stimulation test in early diagnosis of MTC due to the improved sensitivity of sCT assay [17]. In addition, calcitonin can be ectopic produced by neuroendocrine tumors [13]. Thereby, the ability of sCT to command an optimal preoperative evaluation for micro-MTC, is limited.

Ultrasound guided fine-needle aspiration biopsy cytology (FNAB-C) is highly recommended for differential diagnosis of benign thyroid nodule and malignant thyroid neoplasms. However, for MTC, its diagnostic performance may be less convincing, a meta-analysis of fifteen relevant studies indicated FNAB-C can only identify about one-half of all 641 MTC lesions [18]. In a more recent multi-center retrospective study, based on original interpretation alone, the sensitivity of FNAB-C was 68.3% among 145 MTC cases in Asia-Pacific region [19]. The high false-negative rate can be attributed to the morphologic heterogeneity of MTC, and some researchers found micro-MTC has more “mimic” cytopathological features such as less oncocytic change and more colloid presentation [9, 19-21]. Moreover, sampling error of FNAB-C occurs frequently in subcentimeter thyroid nodules [22, 23]. Thereby, the ability of FNAB-C to command an optimal preoperative evaluation for MTC lesions, particular in micro-MTC, is also limited.

Calcitonin assay in fine-needle aspiration biopsy wash-out fluid (FNAB-CT) was firstly described as a diagnostic tool by Boi et al [24]. Thus far, previous studies had indicated FNAB-CT has an ideal diagnostic performance (nearly 100% sensitivity and specificity) and some researchers suggested it as an ancillary tool to confirm inconclusive cytopathologic interpretation [19, 24-32]. However, according to the revised American Thyroid Association (ATA) guidelines for management of MTC, FNAB and the follow up calcitonin measurement in the FNAB washout fluid were recommended only for thyroid nodules that are 1cm or greater in size [33]. The diagnostic performance of FNAB-CT has not been evaluated independently on micro thyroid MTC nodules.
To estimate the diagnostic value of FNAB-CT (versus FNAB-C) in micro thyroid nodules among MTC suspicious thyroid nodules, we conducted a prospective study following the latest STARD statement (STAndards for Reporting Diagnostic accuracy studies) [34]. In consideration of the high sensitivity of basal sCT levels in detecting MTC, MTC suspicious thyroid nodules in the present study were defined as thyroid nodules with elevated basal sCT(≥10pg/ml).

Methods

Study design and participants

A prospective study was conducted from September 2017 to December 2020 in accordance with the STARD statement. The selected patients met at least two criterias: 1) ultrasound examination revealed thyroid nodules. 2) persistent elevated serum calcitonin (sCT≥10pg/ml for more than three months during follow up); The flow of participants through the study was described in Fig1. A total of 78 participants underwent FNAB were consecutively included, 108 thyroid nodules including 57 nodules less than 1cm (defined as micro thyroid nodules) had fine-needle aspiration biopsy performed and wash-out fluid collected for calcitonin assay; 105 thyroid nodules including 56 micro nodules had cytology analyzed. Finally, 60 participants with 87 thyroid nodules met endpoints, including 51 micro thyroid nodules from 39 participants. In addition, peripheral blood samples were taken from 53 participants for RET mutations screening of exons 8, 10, 11, 13, 14, 15 and 16 by standard PCR-based Sanger sequencing.

Hormonal Assay

All tests were performed in a College of American Pathologists (no. 7217913) accredited laboratory. Serum calcitonin (sCT) and FNAB-CT measurement was performed using a chemiluminescent immunoassay (Mindray Medical International, Shenzhen, China) with a sensitivity of 1.0 pg/ml. The inter- and intra-assay CVs for CT were 10 and 5, respectively. Normal range for sCT was 0 to 10 pg/ml. According to the cutoff proposed by Boi et al [24], normal range for FNAB-CT was 0 to 36 pg/ml.

Ultrasonography and FNAB-C

Ultrasound examination was performed using the MyLab ™ Platform ultrasound system (Esoate SpA, Genoa, Italy) equipped with 4-13 MHz linear transducers.

According to thyroid imaging reporting and data (TI-RADS) system described by Kwak et al [35], thyroid nodules were classified into categories with malignancy risks as follows: categories 1 and 2 (0%), 3 (<2%), 4A (2%–5%), 4B (5%–50%), 4C (50%–90%) and 5 (≥90%). The suspicious US features were defined as solid component, hypoechoogenicity, irregular margin, taller than-wide shape and microcalcifications. Thyroid nodule size was measured in three dimensions.

US guide fine-needle aspiration biopsy was performed by certified interventional radiologists using a freehand technique with fine needle (22-gauge generally, 25-gauge for deep-seated or high vascularity lesions) attached to 5-mL plastic syringes. Each target lesion was aspirated two or three times. The obtained material was quickly smeared on glass slides and fixed immediately in 95% ethanol and submitted for haematoxylin-eosin staining.

Cytologic analysis was performed and categorized according to the six-tiered (Category I-VI) Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) [36]. For the purpose of the study, all samples were checked for presentation of morphologic features of MTC. Cytologic diagnose as MTC or MTC suspicious were defined as FNAB-C positive.

SOP for FNAB-CT sample collection and transport

FNAB-CT sample was collected and transported according to standard operating procedures (SOP) as follows:

1. 1ml saline solution was added in a clean EP tube (2.5 ml) and precooled on ice.
2. After smear preparation of specimens for cytologic analysis, the same needle and syringe were quickly washed in precooled saline solution back and forth for at least 5 times.
3. EP tube with collected wash-out fluid were transported to laboratory on ice and centrifuged at 6000g for 10 min at 4°C, supernatant was transferred to new EP tubes for FNAB-CT measurement.
Pathological Analysis

For thyroid nodules submitted to total thyroidectomy, histologic diagnosis including immunohistochemical stains for calcitonin and chromogranin A was performed to differentiate between MTC and non-MTC lesions. All of the histopathologic diagnoses were confirmed independently by two experienced pathologists.

Statistical Analysis

Statistical analyses were performed using SPSS Version 21 and a P <0.05 was considered statistically significant. True positive (TP) and true negative (TN) were defined as the correct prediction of the presentation MTC. The diagnostic performance, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) and overall accuracy were evaluated for FNAB-CT and FNAB-C.

Results

Clinical and demographic characteristics of the patients and lesions

As shown in Fig 1, a total of 108 and 105 thyroid nodules from 78 patients received FNAB-CT and FNAB-C test, respectively. All 57 micro thyroid nodules had FNAB-CT performed and 56 had FNAB-C performed. At the end of the study, 51 micro thyroid nodules from 39 patients and totally 87 thyroid nodules from 60 patients met endpoints: 56 patients underwent thyroidectomy, histology revealed 41 micro-MTC in 32 patients and totally 69 MTC in 52 patients. Eight thyroid nodules (six micro and two macro nodules) from four patients were identified as non-thyroid medullary carcinoma (non-MTC) lesions either had pathologically confirmed pancreatic neuroendocrine tumors (Pnet) with ectopic calcitonin secretion or test error interference by herbal medication.

Clinical and demographic characteristics of 60 patients (28 females and 32 males) are shown in table 1 and table S1. The mean age was 47.90±14.18yrs (range: 23~69yrs), the mean basal level of serum calcitonin (sCT) was 438.06±582.25pg/ml (range: 10.22~2000pg/ml; values higher than 2000pg/ml were calculated as 2000pg/ml). Thirty-nine (65%) patients had at least one micro thyroid nodule with a mean age of 46.50±15.73yrs (range: 23~69yrs); the mean basal sCT was 167.48±352.96 pg/ml (range: 10.22~2000pg/ml). Twenty-two patients (40%) had sCT values ranged from 10 to 100pg/ml; the other 36 patients (60%) had sCT >100pg/ml (sCT values: 48.71±27.93pg/ml vs 559.62±652.15pg/ml; p<0.001). Among 53 patients tested, germline RET mutation was found in 20 patients (16 with micro thyroid nodules) at codons 634(n=15), 618(n=2), 611(n=1) and 533(n=2).

Sonographic characteristics of the 87 thyroid nodules are shown in table 2. The maximum diameter of 87 thyroid nodules ranged from 2.9 to 39mm and 51 (58.62%) were micro nodules (≤10mm), with a mean maximum diameter of 7.23±2.11mm. The majority was in the middle (n=42,48.28%; micro nodules: n=27,52.94%) and middle-upper (n=16,18.39%; micro nodules: n=12,23.53%) area of the lobes. Most of the thyroid nodules were solid (n=84, 96.55%; micro nodules: n=50, 98.04%) and hypochogenicity (n=84, 96.55%; micro nodules: n=51, 100%). Other malignant sonographic features included the presence of microcalcifications (n=38, 43.68%; micro nodules: n=21, 41.18%), irregular margins (n=32, 36.78%; micro nodules: n=18, 35.29%) and tall shape (n=2, 2.30%; both were micro nodules, 3.92%). Following the TI-RADS malignancy risk stratification system, most of the thyroid nodules distributed in TIR4a (low suspicion for malignancy) classification (n=50, 57.47%; micro nodules: n=33, 64.71%) and only 13 of 87 thyroid nodules (micro nodules: 7/51) were categorized as TIR5 (highly suggestive of malignancy) or TIR4c (moderate concern but not classic for malignancy).

Comparison of FNAB-CT with FNAB-C in diagnosing MTC

FNAB-CT were performed on all 87 thyroid nodules. With the cutoff value as 36pg/ml, the sensitivity and specificity was 98.55% and 100%. Nineteen thyroid nodules showed negative FNAB-CT levels and 18 were confirmed as non-MTC lesions. False negative results occurred in a single case of MTC nodule with a size of 9.5*6.2*6.7mm; Sixty-eight thyroid nodules showed positive FNAB-CT level, all were histopathologically confirmed as MTC. The mean value of FNAB-CT in 69 MTC was significantly higher than in 9 non-MTC lesions (1993.40±326.34vs 3.45±2.57pg/ml, p<0.0001).

FNAB-C were performed on 84 thyroid nodules. According to the Bethesda system for reporting thyroid cytopathology (TBSRTC), thyroid nodules were classified into 6 diagnostic categories (DCs): Bethesda I, non-diagnostic/unsatisfactory (ND/UNS) (n=6);
Bethesda II, benign (B) (n=7); Bethesda III, atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS) (n=0); Bethesda IV, follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) (n=26); Bethesda V, suspicious for malignancy (SM) (n=33) and Bethesda VI, malignant (M) (n=12). Sixty-nine nodules including 5 Bethesda I, 4 Bethesda II, 21 Bethesda IV, 27 Bethesda V and 8 Bethesda VI nodules were histologically diagnosed as MTC. Cytologic results as MTC or MTC suspicious were defined as positive. Consequently, 39 lesions were cytologically positive and 38 was histologically confirmed MTC. Among the 45 cytological negative lesions, 31 were diagnosed as MTC in postoperative pathological analysis. Therefore, the sensitivity and specificity of FNAB-C when diagnosing MTC was 55.07% and 93.33%, respectively.

As in table 3, FNAB-CT had a greater performance over FNAB-C when diagnosing MTC regarding as sensitivity (98.55% vs 55.07%), NPV (94.74% vs 31.11%) and overall accuracy (98.85% vs 61.90%).

**Comparison of FNAB-CT with FNA-C in diagnosing micro thyroid MTC nodule**

Subsequently, we explored the performance of FNAB-CT in detecting MTC in micro thyroid nodules. As in table 4, all 51 micro nodules had FNAB-CT performed and 50 had FNAB-C performed. FNAB-CT correctly identified 40 (40/41) micro-MTC but FNAB-C misdiagnosed 21 (21/41) micro-MTC as non-MTC lesions, with diagnostic power as sensitivity (97.56% vs 48.78%), NPV (90.91% vs 30%) and overall accuracy (98.04% vs 58%).

The limited diagnostic accuracy of FNAB-C should ascribe to the high occurrence of false negative diagnosis and micro-MTC had a high false negative rate of 41.18%. As in table 5, among total 31 FNAB-C negative MTC, 21 was smaller than 1cm in size, including four sample error and 17 interpretation error: 12 as follicular neoplasm, three as multinodular goiter, one as PTC suspicious and one as Hashimoto thyroiditis. The other 10 false negative results occurred in macroscopic nodules(>1cm) and included one sample error and nine interpretation error as follicular neoplasm. The majority (30/31) of these FNAB-C negative MTC were revealed by FNAB-CT before surgery.

To be noted, four patients with thyroid nodules (two had only one micro thyroid nodule) and elevated sCT (92.44, 149.71, 467.7, 2000pg/ml) levels were excluded MTC diagnosis based upon FNAB-CT results. Subsequently, two of them found calcitonin-secreting pancreatic neuroendocrine tumors and the other two had normal sCT level after quitting herbal therapy. In addition, two patients with borderline elevated sCT (10.21, 16.43pg/ml) and micro thyroid nodules, received total thyroidectomy and central lymph node dissection due to markedly high levels of FNAB-CT(>2000pg/ml), histopathological findings confirmed MTC in both patients and one with lymph node metastases exist.

**Discussion**

The present study prospectively demonstrated FNAB-CT, with a high diagnostic accuracy, is a reliable tool in detecting MTC before surgery, especially for micro thyroid nodules with elevated serum calcitonin(sCT).

Sensitive ultrasound examination facilitated the early detection of thyroid microcarcinoma including micro-MTC. However, probably to avoid the overtreatment of papillary thyroid microcarcinoma, the current guidelines only recommended thyroid nodules ≥1cm to be evaluated by FNA and cytological diagnosis with or without ancillary tests including calcitonin measured in the FNA washout fluid [33, 37]. For micro-MTC lesions, data are lacking. Previous reports of cases with micro thyroid nodules showed the priority of FNAB-CT over FNAB-C[25, 26, 29]. In the present study, we estimated the two diagnostic tools in micro-MTC independently: FNAB-CT showed better diagnostic performance: sensitivity as 97.56%, specificity as 100%, PPV as 100%, NPV as 90.91% and overall accuracy as 98.04%; whereas FNAB-C established sensitivity and NPV only as 48.78% and 30%, respectively. These data indicated that for thyroid nodules less than 1cm with elevated sCT, FNAB-CT should be performed to exclude micro-MTC.

As neuroendocrine tumor, MTC cells exhibit typical cytologic appearance as eccentrically placed nuclei chromatin (salt and pepper) and usually have a mixed cellular population: mainly spindle-shaped, plasmacytoid or epithelioid cells, and can also present multiple cellular variants, including bizarre giant cells, oncocytic cells, clear cells, and cells with a small cell carcinoma–like appearance. The architectural patterns of MTC are variable as well, ranging from classic discohesive single-cell pattern to cohesive cell fragments. Thus the differential diagnosis for MTC is complicated and should be confirmed by immunolocalization of
calcitonin [20]. When cytologic specimen lacking plasmacytoid morphology and presenting a microfollicular arrangement or round cells with finely granular cytoplasm, MTC can easily mimic follicular lesions. Also, the mixture of normal thyroid follicular cells during sampling contributed to the frequent misinterpretation of MTC as follicular neoplasm [9, 20, 21]. Consistent with previous studies, follicular neoplasm in present study accounted for interpretation error of the majority (21/26) of MTC lesions. We also found micro-MTC tend to have more non-diagnostic results and more variable cytological misdiagnoses. This is reasonable because a subcentimeter size is more challenging in aspiration and resulted in inadequate specimen and intermediate cytological feature [7, 9, 20-23].

Notably, though calcitonin was regarded as the biomarker of MTC and an elevated basal sCT (\(10pg/ml\)) had been proven approximates 100% sensitivity in MTC screening, the PPV was reported only as \(10\%\sim40\%\). As a potentially calcium regulatory peptide, non-MTC derived sCT has been reported from systemic diseases (such as chronic renal failure), pharmacological treatments (such as proton pump inhibitors) and non-MTC malignancies (such as pancreatic neuroendocrine tumors) [13]. Therefore, efforts have been made to enhance the cutoff value to avoid false positive results. Costante G, et al described a 100% PPV for MTC with cutoff of 100pg/ml, but the sensitivity dropped to 60% [12]. Colombo C, et al suggested gender specific cutoff as 18.7pg/ml for female and 68pg/ml for male, obtaining 100% PPV but led to underdiagnosis of two micro-MTC [16]. H Kwon, et al set a cutoff value for macro-MTC as 65 pg/ml to improve diagnostic accuracy but was not able to exclude the presence of micro-MTC [15]. We evaluated the diagnostic performance of FNAB-CT in 29 patients with "intermediate" sCT (10-100pg/ml) and found FNAB-CT exhibited a 100% diagnostic accuracy (versus 61.76% of FNAB-C, tableS2). Indeed, 36.54% (19/52) of the patients with MTC in present study had sCT values ranged from 10 to 100pg/ml, the mean size of their MTC nodules was significantly smaller than MTC nodules with sCT above 100pg/ml (7.59±4.24mm vs 14.32±9.08mm; p<0.001). Furthermore, even significantly elevated sCT are not necessarily associated with MTC. As described before, two participants with thyroid nodules and extremely high levels of sCT (467.7pg/ml and \(\geq2000pg/ml\)) showed negative FNAB-CT results and were proven non thyroid C-cell-derived hypercalcitonemia: ectopic calcitonin-secreting Pnets. Their thyroid nodules were classified as TIR3 or TIR4a on ultrasound examination. Therefore, we suggest thyroid nodule with elevated sCT should be further evaluated by FNAB-CT to exclude the diagnosis of MTC, so that the subsequent appropriate surgical procedures can be determined.

The clinical significance of present study lied mainly in micro-MTC. As reported, the incidence of micro-MTC is growing [2-6]. Unlike micro-PTC, a significant portion of micro-MTC present aggressive clinical feature. In a retrospective study based on SEER database, 37/176 and 5/16/310 patients with micro-MTC showed regional metastases and distant metastases at diagnosis, significantly decreasing their 10-year survival rates [2]. Similar results have been reported by other studies [3, 8]. Two recent studies found MTC patients diagnosed in recent time period had greater proportion of micro-MTC and better clinical outcome [4, 6]. However, size of the tumor alone is not an independent prognostic factor of MTC [10]. Micro-MTC should be managed as macro-MTC. Due to the low specificity of FNAB-C and sCT, we suggested FNAB-CT when a thyroid nodule less than 1cm was suspected of MTC.

In contrast to previous studies [24-28, 30, 31], the characteristic of the present study should be highlighted: 1) first evaluation of FNA-C and FNAB-CT in micro-MTC independently; 2) the prospective design and accordance to STARD statement; 3) detailed description of clinical and demographic characteristics in included patients and lesions. Conversely, we totally included 87 thyroid nodules and 51 micro thyroid nodules, the sample size is a limitation.

In conclusion, we confirmed FNAB-CT as a reliable tool to detecting micro-MTC. When cytology analysis had approximately 50% false negative rate, FNAB-CT kept a nearly 100% diagnostic accuracy. We suggest thyroid nodule less than 1cm with suspected sonographic characteristic should be tested serum calcitonin; for those with elevated sCT, FNAB-CT should be analyzed to exclude the diagnosis of MTC, so that the subsequent appropriate surgical procedures can be determined.

Declarations

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Conflicts of interest:

no conflicts of interest.

Availability of data:

All data generated or analyzed during this study are included in this article.

Code availability:

Not applicable.

Ethics approval:

This study was approved by the board of medical ethics of Ruijin Hospital, Shanghai Jiaotong University.

Consent to participate:

Written informed consents were obtained from all participants included in the study.

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### Tables

**Table 1.** Clinical and demographic characteristics of the 60 patients who meet end-points.

|                      | Total | Patients with micro thyroid nodules | Patients without micro thyroid nodules |
|----------------------|-------|-------------------------------------|---------------------------------------|
| Patients (n)         | 60    | 39                                  | 21                                    |
| Sex (n)              |       |                                     |                                       |
| Female               | 28    | 15                                  | 13                                    |
| Male                 | 32    | 24                                  | 8                                     |
| Age at FNAB-CT/FNA-C yrs |       |                                     |                                       |
| Mean±SD              | 47.90 ± 14.18 | 46.50 ± 15.73  | 49.43 ± 13.06                         |
| Basal sCT pg/ml      |       |                                     |                                       |
| Mean±SD              | 438.06 ± 582.25 | 167.48 ± 352.96 | 761.41 ± 747.68                      |
| Germline RET mutation screening c |       |                                     |                                       |
| Tested               | 53    | 34                                  | 19                                    |
| Not found            | 33    | 18                                  | 15                                    |
| “Hotspot” mutation   | 20    | 16                                  | 4                                     |

*FNAB-CT* calcitonin assay in FNAB wash-out fluid, *FNAB-C* fine-needle aspiration cytology, *sCT* serum calcitonin

a Patients who have micro (≤10mm) thyroid nodules with or without co-existing macro thyroid nodules

b Patients who have no micro (≤10mm) thyroid nodules

c Standard PCR-based Sanger sequencing for RET mutations in exon 8, 10, 11, 13, 14, 15 &16, genomic DNA was extracted from peripheral blood.

**Table 2.** Sonographic characteristics of 87 thyroid nodules.
|                         | Total thyroid nodules | Micro thyroid nodules a |
|-------------------------|-----------------------|-------------------------|
| Number                  | 87                    | 51                      |
| Size (mm)               | 11.94 ± 7.74          | 7.23 ± 2.11             |
|                         | N                     | N                       |
| Distribution            |                       |                         |
| Left lobe               | 38                    | 43.68                   |
|                         | 47                    | 54.02                   |
| Right lobe              | 2                     | 2.3                     |
| Isthmus                 | 2                     | 2.3                     |
| Vertical location       |                       |                         |
| Upper lobe              | 11                    | 12.64                   |
| Middle-upper lobe       | 16                    | 18.39                   |
| Middle lobe             | 42                    | 48.28                   |
| Middle-lower lobe       | 7                     | 8.05                    |
| Lower lobe              | 9                     | 10.34                   |
| Isthmus                 | 2                     | 2.3                     |
| Suspicious sonographic characteristics |                       |                         |
| Solid composition       | 84                    | 96.55                   |
| Hypochogenicity         | 84                    | 96.55                   |
| Microcalcification      | 38                    | 43.68                   |
| Irregular margins       | 32                    | 36.78                   |
| Tall shape (taller than wide) | 2                    | 2.30                    |
| TI-RADS US classification b |                       |                         |
| 3                       | 9                     | 10.34                   |
| 4                       | 50                    | 57.47                   |
| 4a (Low suspicion for malignancy) | 50 | 57.47 | 33 | 64.71 |
| 4b (Intermediate suspicion for malignancy) | 15 | 17.24 | 7 | 13.73 |
| 4c (Moderate concern but not classic for malignancy) | 9 | 10.34 | 6 | 11.76 |
| 5 (Highly suggestive of malignancy) | 4 | 4.60 | 1 | 1.96 |

a Micro thyroid nodule, thyroid nodule has a maximum diameter < 10mm under ultrasound;
b Thyroid imaging reporting and data (TI-RADS) system described by Kwak et al. 33.

Table 3. The diagnostic performance of FNAB-CT, compared with FNAB-C in thyroid nodules.
|     | n  | Positive | TP | FP | Negative | TN | FN | sensitivity | specificity | PPV | NPV | Overall accuracy |
|-----|----|----------|----|----|----------|----|----|-------------|-------------|-----|-----|-----------------|
| FNAB-CT | 87 | 68       | 68 | 0  | 19       | 18 | 1  | 98.55%      | 100.00%     | 94.74% | 98.85% |
| FNA-C   | 84 | 39       | 38 | 1  | 45       | 14 | 31 | 55.07%      | 93.33%      | 97.44% | 31.11% | 61.90% |
| Reference standards | 87 | 69       |    |    | 18       |    |    |             |             |       |       |                 |

FNAB fine-needle aspiration biopsy, FNAB-CT calcitonin assay in FNAB wash-out fluid, FNAB-C fine-needle aspiration cytology, n number of thyroid nodules, Positive MTC positive, Negative MTC negative, TP true positive, FP false positive, TN true negative, FN false negative, PPV positive predictive value, NPV negative predictive value

Table 4. The diagnostic performance of FNAB-CT, compared with FNAB-C in micro thyroid nodules.

|     | n  | Positive | TP | FP | Negative | TN | FN | sensitivity | specificitty | PPV | NPV | Overall accuracy |
|-----|----|----------|----|----|----------|----|----|-------------|-------------|-----|-----|-----------------|
| Micro thyroid nodules a | 51 | 40       | 40 | 0  | 11       | 10 | 1  | 97.56%      | 100.00%     | 90.91% | 98.04% |
| FNAB-CT   | 50 | 20       | 20 | 0  | 30       | 9  | 21 | 48.78%      | 100.00%     | 30.00% | 58.00% |
| FNA-C     | 51 | 41       |    |    | 10       |    |    |             |             |       |       |                 |

FNAB fine-needle aspiration biopsy, FNAB-CT calcitonin assay in FNAB wash-out fluid, FNAB-C fine-needle aspiration cytology, n number of thyroid nodules, Positive MTC positive, Negative MTC negative, TP true positive, FP false positive, TN true negative, FN false negative, PPV positive predictive value, NPV negative predictive value

a Micro thyroid nodule, thyroid nodule has a maximum diameter < 10mm under ultrasound;

Table 5. False negative diagnosis of Fine-needle aspiration biopsy cytology in MTC

| MTC | Micro-MTC | Macro-MTC |
|-----|-----------|-----------|
| False negative counts | 31 | 21 | 10 |
| False negative rate a | 35.63 | 41.18 | 27.78 |
| False negative categories (N) | | | |
| Sample error b | 5 | 4 | 1 |
| Non-diagnostic results | | | |
| Interpretation error c | | | |
| Follicular thyroid neoplasm | | | |
| Multinodular goiter | | | |
| Hashimoto thyroiditis | | | |
| PTC suspicious | | | |

a False negative rate, false negative counts / total counts.
b Adequate cells were not aspirated.
Figures

Figure 1

The flow of the participants through the study.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryTableS1.docx
- supplementaryTableS2.docx