

Case Report

Fine needle aspiration cell blocks in the diagnosis of medullary thyroid cancer: report of four cases and review of the literature

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Abstract: US-Fine needle aspiration biopsy (US-FNAB) is a simple diagnostic procedure to find malignant thyroid nodules. However, few papers have evaluated the specific value of FNAB to detect medullary thyroid cancer (MTC). This study aimed to appraise the value of FNAB cell blocks in the diagnosis of MTC by a novel cell block preparation method. We retrospectively reviewed four typical cases of MTC that were diagnosed with cytomorphology and immunohistochemistry of cell blocks.

Keywords: Medullary thyroid cancer, fine needle aspiration biopsy, cell blocks

Introduction

US-Fine needle aspiration biopsy is a relatively simple, inexpensive, less traumatic and fast diagnostic procedure with high accuracy and efficacy [1, 2]. However, it has some important limitations. For example, in some specimens are not sufficient for definite diagnosis [3, 4]. Ultrasound-guided fine needle aspiration biopsy is able to detect approximately 45-63% medullary thyroid cancer, which indicates that misdiagnosis often occurs with this procedure [5].

Medullary thyroid cancer is a rare, yet clinically significant thyroid carcinoma. It accounts for approximately 5% of thyroid malignancies [1]. It is a well differentiated neuroendocrine carcinoma that arises from parafollicular thyroid cells and produces calcitonin in abundance, leading to a moderate rise in serum level. Although 50% of cases treated with surgical excision recurred, it is so far the primary curative modality [6]. Thus it is important to diagnose MTC before surgery. However, most MTC cases were diagnosed after operation which had missed the optimal treatment time. As a consequence, delay in the diagnosis or incomplete surgical treatment leads to a poor prognosis. New techniques have recently appeared

such as the measurement of calcitonin (CT) in the needle washout (FNAB-CT). Further studies [7-9] showed that FNAB-CT had high sensitivity and specificity in the diagnosis of MTC. However, there is still no unequivocal method for FNAB-CT sampling, or an established cut-off of FNAB-CT for the diagnosis of MTC. FNAB-CT should be considered complementary to FNAB, not as a substitute.

Based on the cytology features of MTC, we processed residual materials from cytological smears in cell blocks to identify MTC. The specimens were composed of random cells and tissue fragments, which can be sectioned for immunohistochemical staining. We have successfully diagnosed four cases of MTC through the morphology and partial histology structures. We then follow up their clinical data relevant to MTC.

Case reports

We selected 4 cases of MTC preoperatively diagnosed by cell blocks from the database of Beijing Friendship Hospital, Department of Ultrasound, between August 2011 and April 2015. They were diagnosed of primary MTC, who had histopathology confirmed MTC and 3 of them accepted thyroidectomy and lymph

Four cases of fine needle aspiration cell blocks in the diagnosis of medullary thyroid cancer

Table 1. Demographic, clinical and biochemical data

Patient	Age	Gender	TSH (μIU/ml)	Tg (IU/ml)	TPO (IU/ml)	CT (pg/ml)	CEA (ng/ml)	Ca (mmol/L)
1	60	F	4.60	N	N	>1000	40.03	N
2	49	F	N	N	N	>1000	129.98	N
3	37	M	N	N	N	>1000	>1000	1.74
4	66	M	N	N	N	>1000	260.33	N

Table 2. Ultrasound features of the nodules

	Size (cm ²)	Location	Nature	Echo	Calcification	Margin	CDFI
1	4.3×2.8	Left	Solid	Hypoechogenicity	Absence	Clear	Absence
2	1.8×1.5	Right	Solid	Hypoechogenicity	Absence	Clear	Absence
3	2.8×1.9	Left	Solid	Hypoechogenicity	Microcalcification	Clear	Presence
4	2.8×1.3	Left	Solid	Hypoechogenicity	Bulky calcification	Unclear	Presence

node dissection. The remaining one had multiple foci when MTC was first discovered. However, the histopathology of bone metastasis offered diagnostic basis.

Ultrasound examination

Ultrasonography was performed using an iU22 ultrasound scanner (Phillips medical Systems Corporation, Netherlands). A complete neck ultrasonographic mapping, including the thyroid, central and lateral neck node compartments, with a high-frequency (10-12 MHz) probe was performed in all patients. According to 2009 ATA guidelines [1], the suspicious malignancy features by ultrasound are: solid aspect, hypoechogenicity, microcalcification, irregular margins.

Cell block preparation procedure

FNAB was routinely performed under US guidance, using a 21G aspirator. Each lesion was aspirated 20-30 times. FNA specimens were collected by direct smear and hematoxylin and eosin (HE) staining. The remaining cells and tissue fragments were processed into cell blocks using 95% ethanol coagulation and formaldehyde fixation. Then the sample was submitted to Department of Pathology. The procedure details were referred to their routine cell blocks procedure [10].

US examinations and US-FNAB were performed by an experienced ultrasound doctor.

Clinical data

Collected clinical data included clinical presentations, ultrasound thyroid examination, CT exam-

ination, laboratory examination (TSH, Tg, TPO, CEA, CT), general data (Tables 1, 2). All the nodes are characterized by isolated low density and presence of reinforcement by CT findings. Finally, patients definitely diagnosed of MTC were followed up to include their prognosis, the level of related markers, and with or without metastasis.

Patient number 1 presented with a swollen neck, no facial flushing and no diarrhea, and had right total thyroidectomy and neck dissection in 2011. Based on the histology and specific stain results, they were diagnosed with definite MTC accompanied with central lymph node resection. During the next four years, she had no local recurrence and metastasis. Likewise, the serum calcitonin, TSH and TG also kept at a relative low level.

Patient number 2 presented with a thyroid neoplasm in the right lobe, no pain, and any clinical symptoms. He had total thyroidectomy and lymph node dissection of central group. The postoperative histology biopsy confirmed of MTC and central lymph node metastasis. During the two years of follow-up, she survived with good shape and no abnormal indicators of recurrence.

Patient number 3 initiated with left thyroid neoplasm. He had right total thyroidectomy and neck lymph dissection in 2013. The postoperative pathology confirmed the presence of MTC without lymph node metastasis. His serum calcium is below the abnormal level. Nevertheless, the level of CT and CEA is far beyond the normal range. He had good prognosis, with no recurrence to now.

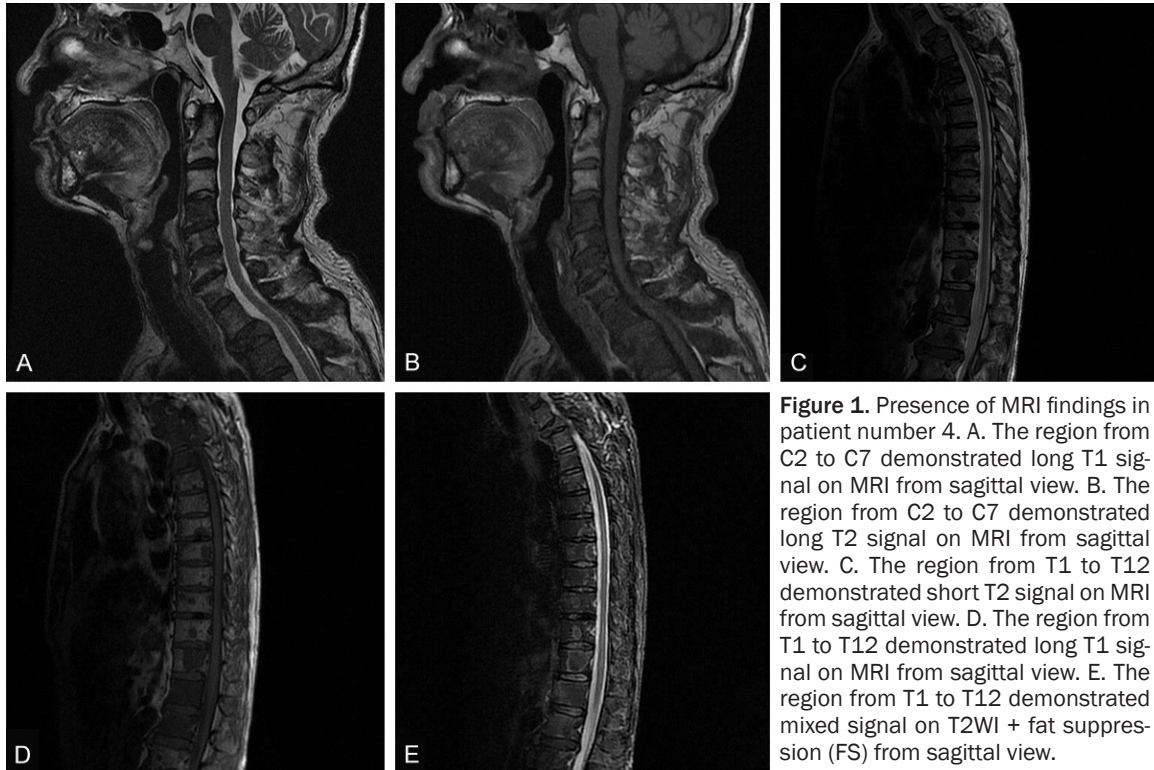


Figure 1. Presence of MRI findings in patient number 4. A. The region from C2 to C7 demonstrated long T1 signal on MRI from sagittal view. B. The region from C2 to C7 demonstrated long T2 signal on MRI from sagittal view. C. The region from T1 to T12 demonstrated short T2 signal on MRI from sagittal view. D. The region from T1 to T12 demonstrated long T1 signal on MRI from sagittal view. E. The region from T1 to T12 demonstrated mixed signal on T2WI + fat suppression (FS) from sagittal view.

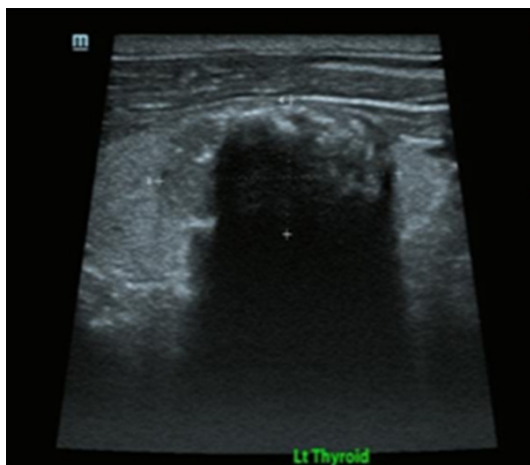


Figure 2. Presence of hypoechogenicity, irregular margin and bulky calcification.

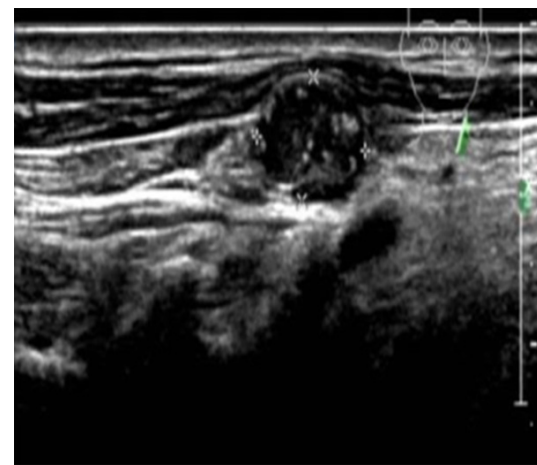


Figure 3. Presence of multiple dotted high echoes in lymph node, indicating the presence of microcalcifications.

Patient number 4 presented with multiple bone pain and couldn't walk at onset. Magnetic resonance imaging (MRI) of the spine revealed abnormal signal from C2-T12 vertebral body and part of the attachment (**Figure 1**). This was suspicious of metastatic tumor or multiple myeloma. Through a series of examinations, a thyroid nodule of the right lobe was discovered. The lesion had some malignant features, such as hypoechogenicity, presence of irregular

margin and bulky calcifications (**Figure 2**). Moreover, there is multiple cervical lymph nodes enlargement combined with calcification (**Figure 3**). We performed an US-FNA procedure to obtain enough specimens to diagnose the disease. Finally, the patient was diagnosed of MTC by the cell blocks immunohistochemistry. Intraoperative thoracic spine lamina and myeloid tissue biopsy supported the conclu-

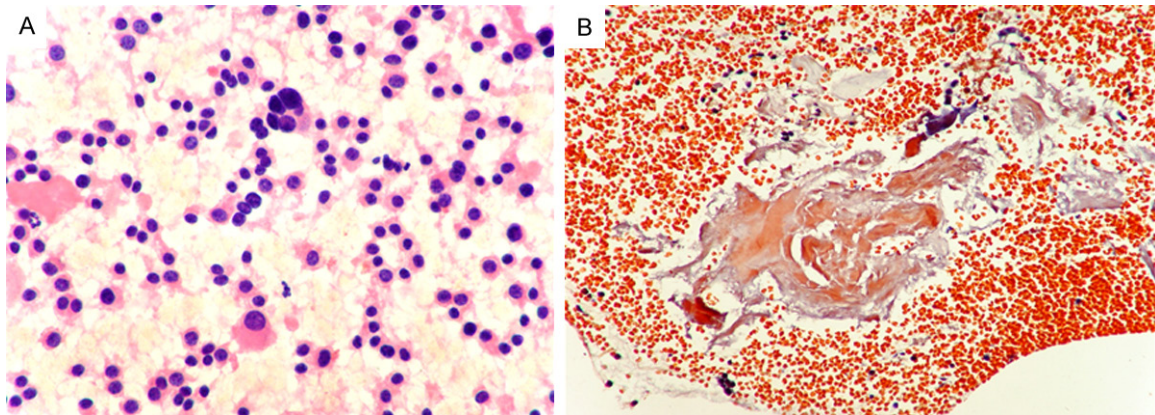


Figure 4. A. A cell smear contained the same size of round-like cells abundant cells which diffusely distribute in the cytoplasm. Occasionally heterotypic large cell exists which is abundant in eosinophilic cytoplasm. Deep-dyed big nucleolus and broadly granular chromatin are also observed without nucleoli. Mononuclear, Dikaryocyte or multi-nuclear cell is usually visible. B. Congo-red positivity presence of amyloid.

Table 3. Expression of related antibodies

	CT	CEA	Syn	CgA	TTF-1	CD56	TG	CD38	CD138
1	+	+	+	+	+	+	-		
2	+	+	+	+	+	+	-		
3	+	+	+	+	+	+	-		
4	+	+	+	+	+	+	-	-	-

sion. CD38 and CD138 are negatively expressed that potentially exclude the diagnosis of multiple myeloma. His clinical symptoms partly relieved after spinal surgery.

Main diagnostic criteria for morphology and immunohistochemistry

The main diagnostic criteria for cytological smears and cell blocks were as follows: 1. high cellularity with single cells or small clusters, absent colloid. 2. A mixture of cell types, such as round or oval, spindle-shaped, and polygonal cells. 3. Amyloid substance (positive at Congo-red staining), homogeneous, in rods or spheres [11]. Previous studies revealed that a reliable confirmation of MTC was obtained by immunocytochemistry with antibodies against calcitonin. Even some studies [7, 8, 12] showed that the measurement of CT in the needle washout (FNAB-CT) had high sensitivity and specificity in the diagnosis of MTC. However, these techniques have certain limitations. Immunocytochemical staining may be difficult in samples with poor cellularity. In the latter procedure tissue structure could not be

obtained and the level of CT could be measured. Further, the importance of other markers was also neglected.

Pathology results

In our study, we performed cell blocks procedure if FNAB cytological smears were suspicious of MTC (Figure 4). A panel of antibodies was selected for immunohistochemical staining (Table 3; Figure 5). However, CD38 and CD138 were also selected for patient number 4 to differentiate from multiple myeloma.

Discussion

Medullary thyroid carcinoma is a rare neuroendocrine tumor associated with hereditary and genetic factors. It makes up 3%-10% all thyroid cancers and 13.4% of all thyroid-related deaths [13]. It occurs as sporadic medullary thyroid carcinoma (SMTC) in approximately 80% of cases and family medullary thyroid (FMTC) carcinoma accounts for 20% [1]. FMTC is part of autosomal dominant genetic disorders, which refers to the mutation of Ret protooncogene located on chromosome 10 [14]. FMTC is usually accompanied by other neuroendocrine tumor, mostly affects bilateral thyroid lobes and polycentric nodes. However, SMTC are generally initiated with a single node in unilateral lobe. In addition, there are no gender differences over morbidity among FMTC cases. Yet SMTC frequently occurs to female ones. Our cases were all sporadic ones, with no other neuroendocrine tumor, and single lesion of unilateral

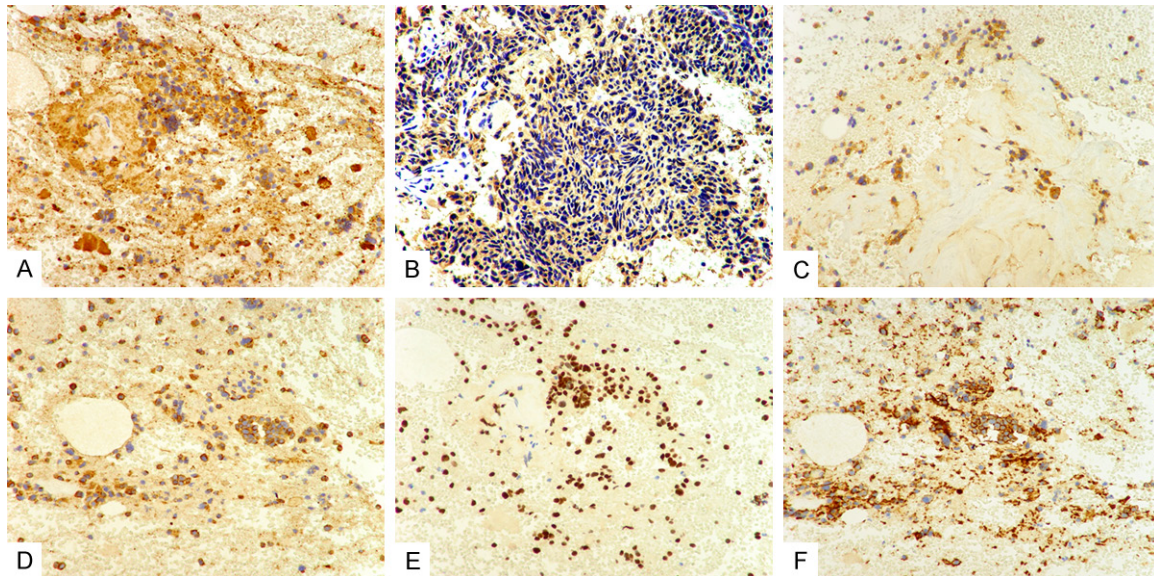


Figure 5. A. Immunohistochemistry of cell block sections revealed CT positivity ($\times 200$). B. Immunohistochemistry of cell block sections revealed CEA positivity ($\times 200$). C. Immunohistochemistry of cell block sections revealed Syn positivity ($\times 200$). D. Immunohistochemistry of cell block sections revealed CgA positivity ($\times 200$). E. Immunohistochemistry of cell block sections revealed TTF-1 positivity ($\times 200$). F. Immunohistochemistry of cell block sections revealed CD56 positivity ($\times 200$).

lobe involvement. These data were in accordance with former studies.

According to the features of the above lesions (**Table 2**), there are no specific ultrasonic findings. Solid and hypoechogenicity lesion is possible of malignancy. However, the value of calcification is less than other malignant tumors. All in all, the diagnostic value of such malignant features is less than that of papillary thyroid cancer. This is the important difference between them, which also leads to missed diagnosis. So we should pay more attention to clinical symptoms (carcinoid syndrome, diarrhea and so on), high level of CT and CEA, which is indicative of MTC and then comprehensively diagnose it preoperatively to get better prognosis.

Generally, MTC is a high malignant degree of thyroid cancers. It sometimes invades the extra thyroid involvement such as trachea, recurrent laryngeal nerve and so on. Even some lesions metastasize to distant parts of the body. Patient number 4 initiated with multiple bone metastasis. Some patients have endocrine disorders, for example, facial flushing and diarrhea, Cushing features, which is the cause of secretion of 5-HT and CT. None of our cases experienced such symptoms. CT is a sensitive

marker of MTC all the time, some investigators regard it as a potent indicator of prognosis and recurrence. Nevertheless, serum calcium mostly keeps at normal range. In our cases, their basal serum calcitonin level > 1000 pg/ml and gradually kept at a normal range after surgery during the follow-up time (**Table 1**). Although the diagnostic value of CT is favorable, there is rare cases of MTC associated with normal serum calcitonin level [15, 16]. In addition, other potential serum markers of MTC (CEA, procalcitonin or other neuroendocrine molecules) have been reported [17]. Machens et al [18] say that patients with poorly differentiated and more aggressive MTC show a rapid CEA increase, usually doubling time. It should be considered as a complementary marker of aggressive MTC during follow-up. However, its baseline determination adds little to the first diagnosis [17]. Our cases just also meet this point. The level of CEA increase from 8-200 times (**Table 1**). Referring to the above markers, there is a debate over whether to use as a routine test, their role in identifying this cancer deserves further studies.

The most reliable diagnostic evidence is still bases on pathological findings and immunohistochemical staining is regarded as golden stan-

dard to diagnose MTC. The tumor cell expresses some important markers, such as CT, Syn, CgA, and CEA. In our study, we found that CT, Syn, CgA, CEA, TTF-1, and CD56 are positive, yet TG is negative in our cell block specimens.

Nowadays, surgical excision is considered to be the first choice to treat MTC. Preoperative diagnosis of MTC predominantly impacts on the prognosis. Although some new diagnostic idea has appeared, such as FNAB-CT and Gallium-68 dotatate PET/CT, cytological examination cannot be replaced because of very high specificity and sensitivity [6, 9]. Yet, there are still rare cases missed. In our study, we use a relatively simple and inexpensive cell block technology to identify special markers and histological morphology.

In conclusion, diagnosis of MTC is complex work. We should combine every method to comprehensively diagnose. Particularly, the clinical, imaging and serum indicators are complementary to the cytological procedure. This study was based on a relatively small sample size. However, it should be considered as a reliable and useful adjunct to FNAB for establishing a definitive MTC diagnosis. More samples should be preserved for future studies.

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Disclosure of conflict of interest

None.

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