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Clinicopathological Features of Calcitonin-Negative Thyroid Neuroendocrine Carcinoma

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Abstract: To clarify the clinicopathological features of calcitonin-negative thyroid neuroendocrine carcinoma. Methods The clinicopathological data of a patient with calcitonin-negative thyroid neuroendocrine carcinoma were retrospectively analyzed, and the clinical, imaging, histopathological and immunohistochemical characteristics were summarized, and the relevant literature was summarized. Results CT showed that the thyroid volume was enlarged and there were multiple low-density shadows in it. Postoperative pathological examination showed that the tumor tissue was solid nested or diffusely arranged, infiltrating the thyroid follicular space, the tumor cells were polygonal, spindle-shaped and oat-shaped, with sparse cytoplasm, easy to see apoptosis and mitotic figures, and large background Coagulative necrosis. Immunohistochemical staining showed positive expression of Syn and CD56 in tumor cells, punctate expression next to the nucleus of a few CK cells, negative expression of CEA, Tg, CgA, calcitonin and TTF-1, and Ki-67 index was about 80%. After 43 months of follow-up, the patient had multiple lung metastases. Conclusion Calcitonin-negative thyroid neuroendocrine carcinoma is rare clinically. This type of tumor is poorly differentiated, positive for immunohistochemical neuroendocrine markers and negative for calcitonin immunostaining. Fine needle aspiration is difficult to confirm the diagnosis and requires a combination of pathologic features and immunohistochemical staining for diagnosis.

1. Introduction

Neuroendocrine tumors of the thyroid are mainly medullary thyroid carcinoma (MTC) of C-cell origin, accounting for only 2-3% of thyroid malignancies [1]. Calcitonin (CT), a product of C cells, has become the most important basis for clinical and pathological diagnosis of medullary carcinoma by serological detection of CT and immunohistochemical detection of lateral CT staining. However, in recent years, the existence of calcitonin-negative thyroid neuroendocrine tumors with pathomorphologic features and immunohistochemical characteristics has made clinical and pathologic diagnosis extremely difficult. Only 16 cases have been reported in the domestic and international literature [2-16], of which only 7 cases [2, 4, 10, 12-14] were diagnosed as calcitonin-negative thyroid neuroendocrine carcinoma. In this paper, we discuss the clinical features, pathological histology and immunohistochemistry, diagnosis, differential diagnosis and prognosis of this carcinoma with the aim of improving the understanding of clinical and pathologists.

2. Materials and Methods

2.1. Data

The patient, a 29-year-old male, was unintentionally found to have a bilateral mass in the thyroid gland for 2 weeks without panic, chest tightness, shortness of breath or weight loss. Specialized examination: a 7.0×2.0 cm tough nodule was found in the right thyroid gland, a 4.0×3.0 cm tough nodule was found in the left thyroid gland, and a 3.0×2.0 cm tough nodule was found in the left side of the isthmus, all of which were poorly defined and mobile, and could move up and down with swallowing. CT scan of the neck and CT scan of the chest showed an enlarged thyroid gland with multiple hypointense shadows and calcified foci in the left lobe; multiple lymph nodes in the neck, some of which were mildly enlarged. The imaging did not reveal any other occupying lesions in the patient. Thyroglobulin, parathyroid hormone and calcitonin were within normal limits. Pathological diagnosis after fine needle aspiration (FNA) suggested a diffuse arrangement of heterotypic cells, which was considered malignant and tended to be of lymphohematopoietic origin. Subsequently, a total radical thyroidectomy and cervical lymph node dissection were performed.

2.2. Methods

FNA specimens were punctured under ultrasound guidance. After local disinfection a 10 ml syringe with a needle (0.8 mm outer diameter) is used to puncture vertically into the thyroid mass and the needle is lifted and inserted more than 10 to 30 times. Until the desired specimen enters the syringe, the needle is withdrawn with a little negative pressure. The puncture specimen in the syringe was sprayed onto the slide with the help of pressure, and the puncture was flattened by using the upper and lower slides, and the slides were pulled apart horizontally to spread the puncture evenly and immediately placed in 95% ethanol for 30 min before routine HE staining. The surgical specimens were fixed in 4% formaldehyde solution, routinely dehydrated, embedded, and serially sectioned 3 μm thick, and then routinely stained with HE and observed by light microscopy. Immunohistochemistry was performed using a fully automated immunohistochemistry instrument (Roche Ventana), and the detection indexes included Syn, Nestin, CD56, CK, desmin, CEA, p63, p53, CD5, CD117, CD99, Oct-4, CD34, CD30, Tg, CgA, calcitonin TTF-1, S-100, PAX-8, LCA, Vim, PGP9.5, LCK, HCK, NSE, CD99, MyoD1, Myogenin and Ki-67, were purchased from Fuzhou Meixin. All primary antibodies were monoclonal antibodies. PBS was used as a blank control instead of primary antibody, and the corresponding antibody-positive tissues were used as positive controls, and the specific assays were performed according to the instrument instructions.

3. Results

3.1. FNA Smear

The tumor cells in the smear were diffusely distributed individually, with little cell cytoplasm, fine nuclear chromatin, pepper salt-like spots, and apoptotic cells and powder-stained necrotic cell debris in the background (Figure 1).

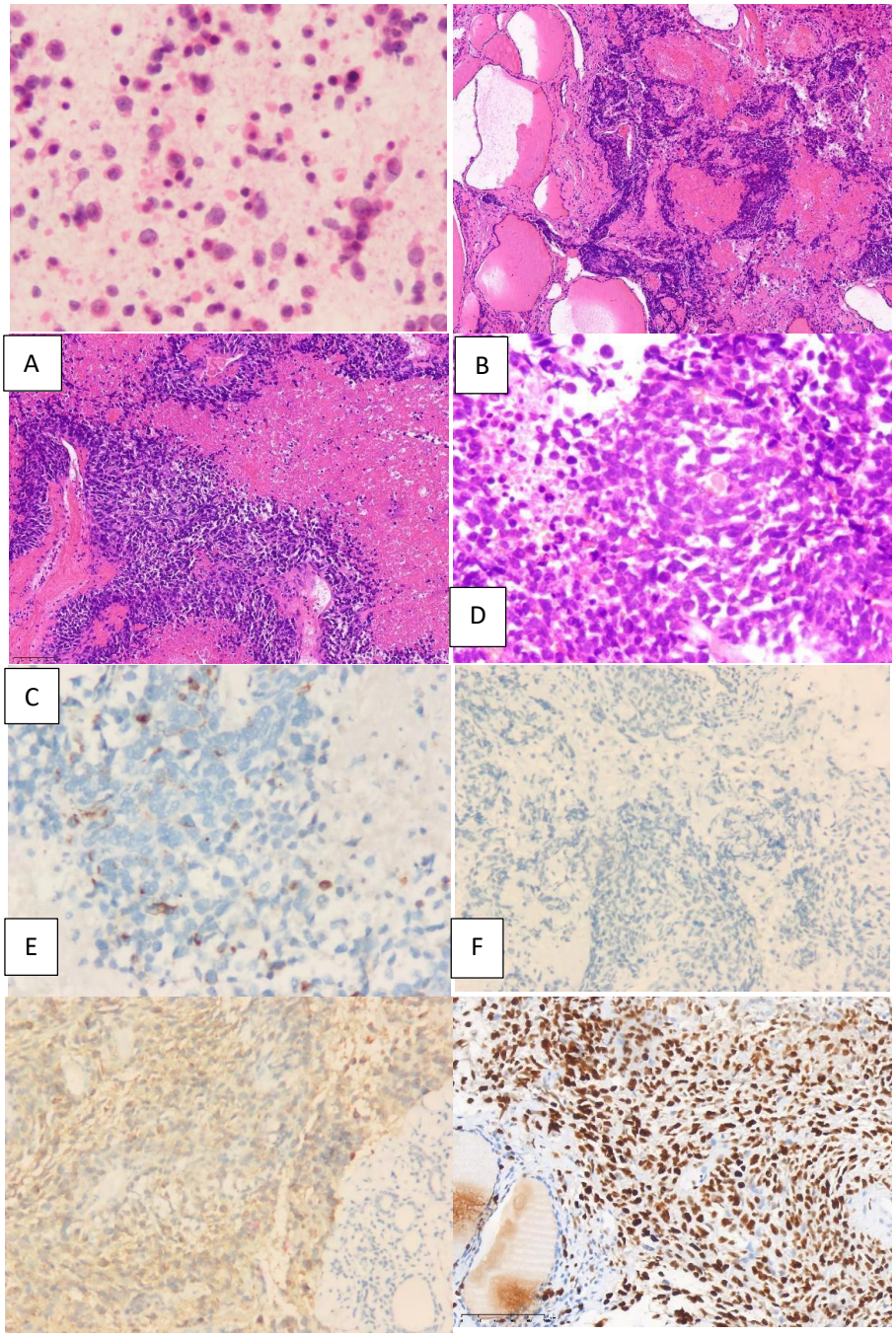

Figure 1: Microscopic features and immunohistochemical expression of calcitonin-negative thyroid neuroendocrine carcinoma

A: The tumor cells in the FNA smear showed a single diffuse distribution, and apoptotic cells and necrotic cell debris were seen in the background (HE ×400). B: The tumor cells in the surgically resected specimen were arranged in irregular nests and infiltrated in the normal follicular space (HE ×40). C: A large area of necrosis was seen in the tumor tissue (HE ×100). D: The tumor cells were polygonal, spindle-shaped and oat-shaped, and the nuclei showed pepper-salt-like changes (HE ×400). E: Immunohistochemistry showed that a few tumor cells were positive for CK adjacent to the nucleus (EnVision ×400). F: Immunohistochemistry showed negative expression of calcitonin in tumor cells (EnVision ×200). G: Immunohistochemistry showed that the cytoplasm of tumor cells was diffusely positive for Syn (EnVision ×200). H: Immunohistochemistry shows that the Ki-67

index of tumor cells is about 80% (EnVision ×200).

3.2. Macroscopic Examination

Thyroid volume 7.5×7×4cm, left lobe volume 7×3.6×3cm, right lobe volume 6×3×4cm, left section gray-red, medium quality, some areas of cystic degeneration with necrosis, volume 6×3×3.5cm, left and isthmus section local gray-red, and cystic degeneration with necrosis, volume 4×3×3cm.

3.3. Microscopic Examination

The tumor cells were relatively uniform in size, solidly nested or diffusely arranged, infiltrating the thyroid follicular space with large areas of coagulative necrosis (Figure 1B and 1C). The tumor cells were polygonal, shuttle and oat-shaped, with sparse cytoplasm, nuclei of about 2-3 small lymphocytes in diameter, ovoid or irregular in shape, fine chromatin, visible fine pepper salt-like spots, inconspicuous nucleoli, some cells extruded and deformed, apoptosis easily seen, and nuclear division images reaching 12-15 cells/2mm2 (Figure 1D). Immunohistochemistry showed positive expression of Syn and CD56 in tumor cells (Figure 1G), CK in few cells (Figure 1E), CEA, p63, p53, CD5, CD117, CD99, Oct-4, CD34, CD30, Tg, CgA , calcitonin (Figure 1F), TTF-1, S-100, PAX-8, LCA Vim, PGP9.5, LCK, HCK, NSE, CD99, MyoD1, Myogenin were all negative, and Ki-67 index was approximately 80% (Figure 1H). Special staining showed negative PAS staining.

3.4. Pathological Diagnosis

Neuroendocrine carcinoma (small cell carcinoma) in left lobe, right lobe and isthmus of the thyroid. 1/33 lymph nodes in the left cervical region IV showed metastasis of cancer tissue, while the remaining 46 lymph nodes in the left cervical region 3, 2 lymph nodes in the left cervical region 6, 24 lymph nodes in the right cervical region 3, 1 lymph node in the right cervical region 4, 9 lymph nodes in the right cervical region 6, 4 lymph nodes in the right cervical region 6B, and 5 lymph nodes in the anterior larynx did not show metastasis of cancer tissue.

3.5. Follow-up

The patient had multiple metastases in both lungs since the date of surgery, with a follow-up period of 43 months up to May 10, 2022.

4. Discussion

The first two cases of calcitonin-negative thyroid carcinoma were reported by Eusebi V [2] in 1990, whose microscopic morphology resembled that of small cell carcinoma with oat cell characteristics and were therefore called calcitonin-negative oat cell carcinoma. one similar case was reported by Chernyavsky VS [3] in 2011 and named calcitonin-negative thyroid neuroendocrine tumor. Mussazhanova Z [4] reported a case in 2014 and named it as calcitonin-negative small cell neuroendocrine carcinoma of the thyroid for the first time. Since then, similar cases have been reported from abroad. Some cases named as "calcitonin-negative MTC" in the literature refer to MTC with serum calcitonin within the normal range and tumor tissue still positive for calcitonin immunohistochemical staining [17]. In the present study, only neuroendocrine carcinoma (NEC) with neuroendocrine features but no evidence of calcitonin production either by serological changes or immunohistochemical staining was investigated, which is a type of calcitonin-negative

neuroendocrine neoplasm (NEN).

Table 1: The clinicopathological summary of caleitonin--negative neuroendocrine neoplasm of thyroid reported in the literatures

Study(year)[Ref]	Patient	Size(cm)	CT	Syn	CgA	TTF-1	TG	CEA	Ki-67	Diagnosis Name	Outcome
Eusebi V (1990) [2]	63y, F	7.0	-	+	+	NA	-	NA	NA	Calcitonin free oat-cell carcinoma of the thyroid gland	Died of disease 1 months after surgery
Eusebi V (1990) [2]	73y, M	5.0	-	+	+	NA	-	NA	NA	Calcitonin free oat-cell carcinoma of the thyroid gland	Died of disease 13 months after surgery
Chernyavsky V S (2011) [3]	40y, F	1.5	-	+	+	NA	+	NA	NA	Calcitonin-Negative Neuroendocrine Tumor of the Thyroid	1 year no clinical evidence of tumor recurrence
Mussazhanova Z (2014) [4]	64y, M	5.4	-	+	+	-	+	-	70%	Small Cell Neuroendocrine Carcinoma of the Thyroid	1 year no clinical evidence of tumor recurrence
Nakazawa T (2014) [5]	76y, M	6.0	-	+	+	+	-	-	<2%	Calcitonin-Free Neuroendocrine Carcinoma of the Thyroid	18 months no clinical evidence of tumor recurrence
Zheng GT (2014) [15]	51y, M	3.0	-	+	+	NA	-	NA	<1%	calcitonin--negative neuroendocrine tumor of thyroid	1 year no clinical evidence of tumor recurrence
Ismi O (2015) [6]	57y, M	15	-	+	+	-	-	-	70%	Calcitonin-negative neuroendocrine tumor of thyroid	NA
Kim GY (2015) [7]	34y, M	0.6	-	+	+	+	+	-	NA	Calcitonin-negative neuroendocrine tumor of thyroid	1 year no clinical evidence of tumor recurrence
Kasajima A (2016) [8]	48y, F	2.8	-	+	+	+	-	-	0.3%	Calcitonin-negative neuroendocrine tumor of thyroid	NA
Parmer M (2017) [9]	74, F	1.6	-	+	+	+	-	+	1%	Calcitonin-negative neuroendocrine tumor of thyroid	NA
Chorny JA (2018) [10]	42, F	6.5	-	+	-	+	-	-	90%	calcitonin-negative neuroendocrine carcinoma of the thyroid	5 year no clinical evidence of tumor recurrence
He J (2018) [16]	52, F	0.6	-	+	+	+	-	+	<1%	Calcitonin-negative neuroendocrine tumor of thyroid	2 year no clinical evidence of tumor recurrence
Cai HJ (2020) [11]	56, F	2.6	-	+	+	+	-	-	20%	Calcitonin-negative neuroendocrine tumor of thyroid	3 months later metastases in the liver
Montgomery G (2020) [12]	45, M	2.5	-	+	+	+	NA	+	NA	Calcitonin negative medullary thyroid cancer	NA
Murphy DC (2020) [13]	24, F	3.5	-	+	+	+	-	-	8%	Calcitonin-negative medullary thyroid carcinoma	3 year no clinical evidence of tumor recurrence
Fernández-Ferreira R (2021) [14]	33, M	4.7	-	+	-	+	-	-	90%	Calcitonin-Negative Neuroendocrine Carcinoma of the Thyroid	showed a complete response 17 months after diagnosis

Abbreviations: CT,calcitonin; Tg, thyroglobulin; CEA, carcinoembryonic antigen; Syn,synaptophysin ;CgA,chromogranin-A;TTF-1, thyroid transcription factor-1; F, female; M, male; NA, not available.

Sixteen cases of calcitonin-negative thyroid NEN are known to be reported in the literature, 8 in men and 8 in women, with a mean age of 50.6 years and a mean tumor diameter of 4.4 cm (Table 1). Immunohistochemical staining was negative for calcitonin and positive for at least 2 of the neuroendocrine markers Syn, CgA and CD56. TTF-1 and Tg as markers of thyroid follicular epithelial

origin were variably expressed, while CEA, which is usually positively expressed in MTC, was expressed in only 3 cases. Referring to the 5th edition of the WHO classification of tumors of the digestive system [18], NEN were classified into neuroendocrine tumor (NET) and NEC, with NET having mild, well-differentiated tumor cells and NEC having poor differentiation. Seven cases in Table 1 were consistent with the diagnosis of NEC based on morphological features, and the remaining cases were consistent with the diagnosis of NET. The tumor cells in the cases reported in this study were sparse in cytoplasm, medium-sized nuclei, and poorly differentiated, consistent with small cell type NEC, which is the first case reported in China. The Ki-67 proliferation index of these eight NEC cases ranged from 8 to 90%, and two of them, including the present case, had distant metastases [11]. In contrast, the Ki-67 index was less than 2% in all cases consistent with NET, and no distant metastasis was seen at follow-up. This suggests that Ki-67 index is closely related to tumor differentiation and prognosis. The origin of thyroid calcitonin-negative NEN is still controversial, and Nakazawa T et al [5] reported a case of calcitonin-negative thyroid neuroendocrine tumor with positive expression of calcitonin gene-related peptide (CGRP) although the tumor cells were negative for calcitonin expression by immunohistochemistry. However, Murphy DC [13] reported a case with negative calcitonin and CGRP immunohistochemistry and mRNA in situ hybridization, as well as a negative MTC-associated RET gene mutation, suggesting that such tumors may not be of C-cell origin.Mussazhanova Z [4] performed TTF-1, thyroid stimulating hormone (TSH) receptor and thyroid hormone (TSH) receptor mutations on a thyroid calcitonin-negative NEC he reported. TSH) receptor and thyroglobulin by reverse transcription-polymerase chain reaction (RT-PCR) analysis, which confirmed the presence of thyrotropin receptor (TSHR) and Tg expression at the transcriptional level, providing evidence for the follicular cell origin of this tumor.

The differential diagnosis of calcitonin-negative thyroid neuroendocrine carcinoma needs to be made with the following tumors (1) Ewing sarcoma/primitive neuroectodermal tumors (Ewing sarcoma/PNET): primary thyroid PNET is extremely rare and difficult to confirm by FNA aspiration cytology [19]. Microscopically tumor cells were arranged in small blue round cells. Tumor cells may be positive for Syn and CgA and negative for CEA and calcitonin. In addition, CD99, S-100 and PAS are usually diffusely positive, whereas CD99, S-100 and PAS are negatively expressed in calcitonin-negative thyroid NEC. (2) Paraganglioma: Tumor cells consisted of plasma cell-like cells and epithelial-like cells in a nested, trabecular arrangement. Tumor cells were positive for CgA, CD56, and Syn, while negative for cytokeratin, calcitonin, CEA, thyroglobulin, and TTF-1, and tumor cells were positive for S-100 in supporting cells [20]. Calcitonin-negative thyroid NEC tumor cells were poorly differentiated and irregularly arranged, with negative expression of S-100, which can be differentiated by combining HE microscopic morphology and immunohistochemistry. (3) Hypofractionated thyroid carcinoma: The tumor tissues are solid, beam-like or island-like, with infiltrative growth, accompanied by necrosis and vascular infiltration, and positive expression of Tg, PAX-8 and TTF-1, and negative expression of Syn, CgA and CD56. (4) Lymphopoietic system tumors: In FNA smear, tumor cells in lymphopoietic system diseases are often diffusely arranged as a single tumor, and there are often no obvious large nucleoli, and necrosis is easily seen. They can be identified by immunohistochemical staining with the help of cell blocks [21], which have negative expression of CK, Sgn, and CgA and positive expression of LCA. (5) Metastatic neuroendocrine carcinoma: Neuroendocrine carcinoma of the lung and gastrointestinal tract may also metastasize to the thyroid gland, especially tumor cells of pulmonary origin often express TTF-1 positively [22, 23]. It needs to be combined with imaging findings to exclude other parts of the body as primary, which can be diagnosed as thyroid primary.

5. Conclusion

Thyroid calcitonin-negative neuroendocrine carcinomas are rare clinically, and their preoperative serum calcitonin and CEA are within normal limits. These tumors are poorly differentiated, positive for immunohistochemical neuroendocrine markers and negative for calcitonin immunostaining. The origin of the tumor is unclear and currently favors a follicular epithelial origin. Fine needle aspiration is difficult to confirm the diagnosis and requires a combination of pathologic features and immunohistochemical staining for diagnosis.

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