Conventional Papillary Thyroid Carcinoma With Intralobular Lymphatic Dissemination Shows More Aggressive Features

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Abstract

Objective: To investigate the invasive capability and other clinicopathological features of conventional papillary thyroid carcinoma (CVPTC) with intralobular lymphatic dissemination.

Methods: Seventy-three CVPTC patients receiving total thyroidectomy were analyzed in this study. The expression of BRAF-V600E, D2-40 and CD31 in all thyroid samples was detected by immunohistochemical staining (IHC). The results were evaluated by two pathologists and were statistically analyzed. In addition, the rate of positive BRAF-V600E expression and the clinical invasiveness of CVPTC with intralobular dissemination (ID-CVPTC), multiple primary CVPTC (MP-CVPTC) and single focus CVPTC (SF-CVPTC) were evaluated. The correlation between BRAF-V600E expression, lymphatic vessel density (LVD), microvessel density (MVD) and the clinicopathological characteristics of CVPTC were assessed.

Results: Twenty-five ID-CVPTC, 17 MP-CVPTC and 31 SF-CVPTC cases were included in this study. The positive expression rate of BRAF-V600E in ID-CVPTC (92.0%) was significantly higher than that in MP-CVPTC (70.6%) and SF-CVPTC (71.0%), while no significant difference in expression between MP-CVPTC and SF-CVPTC was detected (P > 0.05). The expression of BRAF-V600E was not related to clinicopathological features, including age, gender, lymph node metastasis (LNM), bilateral involvement, presence of vascular tumor thrombus, capsule invasion, nerve invasion or the maximum tumor diameter (P > 0.05). The LVD in the ID-CVPTC group (9.74 ± 2.98) was higher than that in the non-ID-CVPTC group (7.46 ± 2.5) (P < 0.05). Compared with cases without adenolobar dissemination, ID-CVPTC was associated with a younger age, higher LNM rate, and increased capsule and vessel invasiveness (P < 0.05).

Conclusions: ID-CVPTC shows more aggressive features, and intralobular lymphatic dissemination may be a potential biological indicator of poor prognosis.

Introduction

With the popularization of physical examinations and changes in the environment and diet, the incidence of thyroid cancer has increased dramatically and ranks as the fourth most prevalent carcinoma in China, followed by breast cancer, lung cancer and colorectal cancer. Conventional papillary thyroid carcinoma (CVPTC) is the most common thyroid cancer, accounting for 80% of all cases. The overall prognosis of CVPTC is satisfactory; the 5-year survival rate is nearly 98.6%, and the 15-year disease-free survival rate is 87.4%. However, the long-term recurrence rate is still high (> 30%), and the mortality is as high as 8%. The prognosis-related factors include gender, age, tumor size, multifocal status, and lymph node metastasis. Numerous studies on the mechanism underlying CVPTC occurrence, development, metastasis and recurrence have made great efforts to identify the key indicators predicting poor prognosis in CVPTC and more effective therapeutic targets. However, no clear conclusion has been obtained. Multiple foci have been detected in approximately 35% of CVPTC cases, and we found
obvious pathological changes in terms of the histological morphology and biological behavior of CVPTC with multiple foci in clinical practice. Such lesions, which showed intralobular spread in the thyroid gland, were named CVPTC with intralobular dissemination (ID-CVPTC). Some studies have suggested that the BRAF-V600E mutation is a predictor of the increased invasiveness of tumors \(^6\), and immunohistochemical results showed good consistency with the results of polymerase chain reaction (PCR) and gene sequencing \(^7\). In this paper, the expression of BRAF V600E, invasiveness and clinicopathological characteristics of ID-CVPTC were investigated to generate additional supportive information for grading the clinical risk.

**Materials And Methods**

1. **Materials**

1.1 Patients and clinical data. Seventy-three CVPTC specimens were collected from the Department of Pathology, Shenzhen Hospital, Cancer Hospital, Chinese Academy of Medical Sciences, from January 2018 to December 2019. The patients were aged from 15 to 62 years old with a median age of 37 years and included 25 males and 48 females. Among the 73 cases, 56 cases involved cervical regional lymph node metastasis (76.7%). None of the patients were treated with any preoperative therapies such as radiation, chemotherapy, endocrine therapy or immunotherapy before diagnosis, except patients with other malignant tumors.

1.1.2 Immunohistochemical analysis. The main reagents were rat anti-human BRAF monoclonal antibody and enhanced antibody II (Roche Diagnostic Products, Shanghai, China). Rat anti-human monoclonal antibody D2-40/CD31, general antibody II, phosphate buffer, 9.0, EDTA antigen repair solution, the DAB color development kit, and other reagents were obtained from Maixin Biotechnology Development Co., Ltd. (Fuzhou, China).

1.2 Methods

1.2.1 Inclusion criteria. The biopsies of CVPTC cases were re-examined by two pathologists under a microscope, and the patients were divided into groups according to whether intralobular dissemination was observed under the microscope (Fig. 1).

In CVPTC specimens, the follicular epithelium showed papillary and branching hyperplasia, fibrous vascular bundles, and interstitial fibrosis and was covered with tumor cells on the surface. Under a high-power microscope, the tumor nuclei were enlarged, elongated, oval-shaped, and arranged in a crowded and overlapping pattern, and they showed a ground glass-like morphology without nucleoli or nucleoli that were clinging to the nuclear membrane; the nuclear membrane was irregular (with the appearance of nuclear pseudoinclusions and nuclear grooves) (Fig. 2A). In terms of the standard for intralobular dissemination, multiple small CVPTCs were found in the surrounding and adjacent thyroid tissues of CVPTC. The ID-CVPTC boundary was not clear, and the tumor cells had spread into and invaded the normal thyroid tissue (Fig. 2B). The normal arrangement of thyroid follicles was lost, and in the normal
thyroid tissues that were at least 0.5 cm away, multiple small follicular epitheliums were observed to form a cavity-like carcinoma nest (Fig. 2C). At high magnification, the tumor cells showed ground glass-like nuclei with abundant cytoplasm (Fig. 2D).

1.2.2 Immunohistochemistry. Formalin-fixed (10%), paraffin-embedded tumor tissue sections were cut sequentially at a thickness of 4 μm. Immunohistochemical staining was performed by following the UIP method. In brief, paraffin sections were dried in a 75°C oven for 15-20 min, dewaxed with xylene, hydrated with a high-to-low alcohol gradient, and washed with distilled water 3 times for 1 min each. Then, the sections were immersed in Tris/EDTA buffer (D2-40/CD31, pH 8.0; BRAF V600E, pH 8.0) and repaired under high temperature and pressure, after which the slides were cooled for 1.5-2 h for antigen retrieval. After washing with distilled water 3 times for 1 min each, 3% H2O2 was added to eliminate endogenous peroxidase, and the slides were incubated in a wet box at room temperature for 15 min. PBS (phosphate buffer, pH 7.5) was used to wash the sections 3 times for 1 min each, and the primary antibody (either D2-40: Fuzhou Maixin, clone number D2-40, anti-human mouse monoclonal antibody working solution; CD31: Fuzhou Maixin, clone number MXO32, anti-human mouse monoclonal antibody working solution, or BRAF V600E: Shanghai Roche, clone number: VE1, anti-human mouse monoclonal antibody working solution) was added 15 min before incubation; then, the slides were incubated in a wet box at room temperature (D2-40/CD31 for 1.0 h; BRAF-V600E for 2 h). The slides were washed with PBS 3 times for 1 min each and incubated with an enhanced secondary antibody at room temperature for 15 min, which was followed by washing with PBS 3 times for 1 min each. Then, the slides were stained with DAB. After hematoxylin staining, the sections were dehydrated and finally sealed with neutral gum.

1.3 Assessment. The immunohistochemical results were evaluated by two pathologists in a double-blind setting. The positive staining of the BRAF V600E protein revealed brown yellow granules located in the cytoplasm. Ten visual fields were randomly selected to calculate the percentage of positive cells under a high-power (400×) microscope. No staining or a number of positive cells < 5% was regarded as negative (-), and a number of positive cells > 5% was regarded as positive (+). The positive expression of D2-40/CD31 was observed in the cytoplasm or cell membrane of lymphatic endothelial cells, which also showed brown-yellow or brown granules. The quantification of D2-40 (LVD)/CD31 (MVD) was executed according to the second international consensus on the angiogenesis quantitative method and standard for solid tumors proposed by Weidner, Vermeulen PB and Bono. The vascular density area was oriented under a 200× light microscope, and the number of vessels in 5 visual fields was counted, the average of which was referred to as the LVD/MVD; the lumen form (which can be in a closed state) was the unit used for counting.

1.4 Statistics. Data were analyzed with SPSS ver. 25.0 (SPSS Inc., Chicago, IL, USA). Pearson's chi-square test and Fisher's exact probability method were used to analyze the significant differences in BRAF-V600E among the groups. The relationship between the LVD, MVD and CVPTC clinicopathological features and grouping was analyzed by a t-test with two independent samples. A P-value <0.05 was considered significant.
Results

2.1 Case grouping

Among the 73 cases, 25 cases of ID-CVPTC comprised the experimental group, 17 cases of MP-CVPTC comprised the control group, and 31 cases of SF-CVPTC comprised the blank control group. The specific clinical and pathological characteristics are shown in Table 1.

Table 1. Patient demographics and tumor pathological features

2.2 BRAF-V600E expression

BRAF-V600E expression was higher in ID-CVPTC (92.0%) than in MP-CVPTC (70.6%) and SF-CVPTC (71.0%). The expression of BRAF-V600E was significantly associated with intralobular dissemination (P < 0.05) but was not related to other clinicopathological features, including age, gender, LNM, bilateral involvement, vascular tumor thrombus, capsule invasion, maximum tumor diameter, number of foci, Hashimoto's thyroiditis (HT) status, psammoma bodies or nerve invasion (P > 0.05) (Table 2 and Figure 3).

Table 2 Relationship between BRAF-V600E protein expression and clinicopathological parameters

2.3 D2-40 and CD31 could identify vessels

In PTC, lymphatic microvessels with positive staining of D2-40 had thin walls and irregular shapes and were rare in the tumor but were prevalently distributed in the adjacent normal tissues, and the LVD was determined. CD31-positive microvessels were arrayed within cords or lumen with an irregular shape and an uneven distribution. The endothelial cells in areas of neovascularization were arranged irregularly and were densely distributed in the tumor (fiber vessel axis of the PTC papillary structure). The MVD of microvessels adjacent to carcinoma tissue was determined. (Fig. 4)

2.4 LVD and MVD determination

The LVD in the ID-CVPTC group (9.74 ± 2.98) was higher than that in the non-ID-CVPTC groups (7.46 ± 2.5) (P < 0.05), including the MP-CVPTC and SF-CVPTC groups. The LVD was not statistically correlated with clinicopathological features, including the foci number, maximum tumor diameter, LNM, gender, bilateral involvement, vascular tumor thrombus, nerve invasion, or BRAF-V600E expression (P > 0.05) but was correlated with age and capsule invasion (P < 0.05) (Table 3).

Table 3 Relationship between the LVD, MVD and PTC clinicopathological features

2.5 Vascular tumor thrombus

In the ID-CVPTC group, tumor thrombi in lymphatic vessels could be found in 8 cases after staining, indicating that the route of intralobular dissemination may be through lymphatic vessels (Fig. 5).
2.6 The difference in the clinicopathological features between ID-CVPTC and non-ID-CVPTC patients

The age of ID-CVPTC patients (34.56 ± 2.44) was younger than that of non-ID-CVPTC patients (41.13 ± 1.44) (P < 0.05); the rate of LNM (96%) in ID-CVPTC patients was higher than that in non-ID-CVPTC patients (66.7%) (P < 0.05). The maximum diameter of ID-CVPTC (the primary focus) was often larger than 1 cm, which was significantly higher than that of non-ID-CVPTC (P < 0.05). In addition, significant differences in capsule and vascular invasion were found in these two groups (P < 0.05), indicating the more invasive behavior of ID-CVPTC. In terms of microscopic morphology, the occurrence of disseminated psammoma bodies in glandular lobes was increased in ID-CVPTC patients (P < 0.05) (Table 4).

Table 4 Relationship between intralobular dissemination and the clinicopathological parameters

Discussion

Over the past few decades, various studies have confirmed that multifocality is an independent risk factor for cervical LNM derived from CVPTC, but the long-term prognostic significance is still unclear. As a result of multifocal CVPTC, intralobular dissemination was observed in clinical practice. The factors related to the invasiveness of CVPTC were studied to provide additional valuable information for clinical risk classification.

BRAF is the main component of the mitogen-activated protein kinase signaling pathway and is well documented as the classical downstream molecule of the Ras and RET proto-oncogenes. Kinase overexpression and BRAF gene mutations dysregulate cell growth, reproduction, invasion and apoptosis. The detection rate of BRAF-V600E in PTC is as high as 79%\(^{11}\), and the results of immunohistochemistry were consistent with those of PCR and gene sequencing. There have been many studies focusing on BRAF mutations and PTC metastasis and prognosis, some of which have suggested that the presence of BRAF-V600E mutations in PTC is related to the subtype but not LNM\(^{12}\). Additionally, BRAF-V600E mutations are often accompanied by increased invasiveness\(^6\). In this study, the rate of BRAF-V600E mutations was 78% (57/73). No correlation was found between BRAF-V600E mutations and the clinicopathological features, including age, gender, LNM, bilateral involvement, vascular tumor thrombus, capsule invasion, foci number, nerve invasion or maximum tumor diameter, while BRAF-V600E expression was significantly correlated only with intralobular dissemination in this study (P < 0.05). Our finding was not consistent with that of the study by Pessoa, which demonstrated the independent correlation of BRAF-V600E with age and HT\(^{13}\).

Patients with LNM tend to have rapid disease progression and an increased local recurrence rate, invasive ability and even mortality, which are important factors affecting prognosis\(^{14}\). CD31 is a vascular endothelial marker normally used to evaluate the tumor MVD. D2-40 is a marker of lymphatic endothelial cells, which can be used to quantify the LVD. Lee and other scholars have suggested that the LVD instead of the MVD is positively correlated with LNM\(^{15}\). Similarly, our study showed that the LVD in PTC patients...
with LNM was higher than that in PTC patients without LNM, and the LVD in ID-CVPTC patients was significantly higher than that in non-ID-CVPTC patients (p < 0.05). However, some other studies have shown that the LVD is not related to the LNM, and the ability of the tumor itself to invade lymphatic vessels is more influential than the stimulatory effect of PTC lesions on promoting new lymphatic vessel generation in peritumoral tissues when LNM appears\textsuperscript{16}. In this study, there were no lymphatic vessels but abundant blood vessels in the tumor, which was consistent with the morphology of the fibrous vascular axis of the PTC papillary structure. The proportion of vascular tumor thrombi in ID-CVPTC cases was increased, confirming that this phenomenon was associated with increased invasive ability.

Due to the popularity of physical examination, the detection rate of thyroid nodules ranges from 19% to 67\%\textsuperscript{17}, and the PTC incidence is dramatically elevated, suggesting that the poor prognosis of PTC is very important in PTC patient management. Bansal reported that multifoci PTC is more likely to lead to multiple synchronous primary tumors (MSPTs) through gene detection\textsuperscript{18}. There were 2-4 foci in the selected multifoci cases, and different subtypes (CVPTC, follicular subtype, high cell type, etc.) were selected. The cases with small tumor-infiltrating foci around large tumors had the same gene alterations, which were similar to those in the intraglandular dissemination foci proposed in this study rather than those in independent primary foci. The results of this study showed significant differences in BRAF-V600E expression and MLD between ID-CVPTC and non-ID-CVPTC patients, while no significant difference was observed between MP-PTC and SF-PTC patients, suggesting that the presence of multiple primary lesions did not affect the invasiveness of CVPTC; multifoci patient prognosis was similar to that of patients with a single focus. Therefore, MP-PTC and SF-PTC patients were grouped into one group, the non-ID-CVPTC patient group, which was used as the control versus the ID-CVPTC patient group, demonstrating that increased invasiveness of PTC could be characterized by intralobular lymphatic dissemination. In terms of BRAF-V600E expression, the p value of the comparison between MP-CVPTC and ID-CVPTC was nearly 0.5, indicating a possible significant difference. However, further study of additional cases is still needed. Compared with patients with nonintralobular dissemination, patients with ID-CVPTC were younger. Such lesions manifest unique microscopic morphological features; the tumors form small follicular epithelium in normal thyroid tissue, and the tumor cells contain ground glass-like nuclei and abundant cytoplasm under a high-power microscope. The morphological difference between PTC and diffuse sclerosing variant PTC (DSV-PTC) is the lack of extensive fibrosis, the large number of psammoma bodies and diffuse manifestations of Hashimoto’s thyroiditis. Although the background lymphocyte infiltration and the probability of psammoma body appearance in the ID-CVPTC group were higher than those in the non-ID-CVPTC group, the pathological changes of the two groups were still different. RET/PTC rearrangement in DSV-PTC was common, while BRAF mutations were rare.

The route of intralobular dissemination may be through lymphangiosis. Although there were only 8 cases (32\%, 8/25) with vascular tumor thrombus in the ID-CVPTC group, the number of cases was higher than that in the non-ID-CVPTC group. We speculated that the reason may be the thin lymphatic vessel wall, which was not easy to observe in formalin-fixed specimens. In this study, there was a correlation between LVD and intralobular dissemination, which was higher than that observed in the non-ID-CVPTC group.
Whether intraglandular spread occurs via lymphatic vessels needs to be confirmed by including additional cases.

In conclusion, ID-CVPTC is more commonly diagnosed in young women and shows increased invasiveness. In this study, only 25 ID-CVPTC cases were collected and studied at the morphological and protein level. Further studies on gene detection, family aggregation and long-term follow-up in a larger cohort are needed. The aim of this work is to convince pathologists to pay attention to this type of special case in pathological diagnosis and to provide more reference information for clinical risk classification.

**Abbreviations**

CVPTC: conventional papillary thyroid carcinoma; DSV-PTC: diffuse sclerosing varian PTC; HT: Hashimoto thyroiditis; ID-CVPTC: CVPTC with intralobular dissemination; IHC: immunohistochemical staining; LNM: lymph node metastasis; LVD: lymphatic vessel density; MP-CVPTC: multiple primary CVPTC; MVD: microvessel density; PCR: polymerase chain reaction

**Declarations**

**Acknowledgements**

Not applicable.

**Author Contributions**

Yuanyuan Lei: contributed significantly to the analysis and manuscript preparation;

Wenting Huang contributed to the conception of the study. Qiuxiao Yu wrote the manuscript. Sha Feng performed the data analyses. Guihua Shen contributed reagents/materials/analysis tools. Lijuan Yuan: contributed reagents/materials/analysis tools.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

This study was approved by ethics committee of Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (Shenzhen, China). Written informed consent was also obtained from all patients.
Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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**Tables**

**Table 1.** Patient demographics and tumor pathological features

|                      | ID-CVPTC(n=25) | MP-CVPTC(n=17) | SF-CVPTC(n=31) |
|----------------------|----------------|----------------|----------------|
| **Age, years**       | 34.56±12.20(15-58) | 44.59±9.67 (31-62) | 39.23±9.75(25-62) |
| **Sex, M:F**         | 10:15          | 4:13           | 11:20          |
| **Largest tumor diameter, cm** | 1.32±0.62(0.2-0.8) | 1.11±0.77(0.4-3.5) | 1.55±0.98(0.2-3.5) |
| **Bilateral: unilateral** | 11:14         | 16:1           | 31:0           |
| **TNM staging**      | 2:8:15         | 4:5:8          | 13:11:7        |
| **LNM, +/−**        | 24:1           | 13:4           | 19:12          |

**Table 2** Relationship between the BRAF-V600E protein expression and clinicopathological parameters
| Variables                      | Total cases n= | BRAF+ n= | BRAF- n= | $\chi^2$ | P   |
|-------------------------------|----------------|----------|----------|-----------|-----|
| age/year                      |                |          |          |           |     |
| ≤40                           | 49             | 36       | 13       | 1.853     | 0.173|
| ≥40                           | 24             | 21       | 3        |           |     |
| gender                        |                |          |          |           |     |
| man                           | 25             | 20       | 5        | 0.082     | 0.775|
| female                        | 48             | 37       | 11       |           |     |
| LNM                           |                |          |          |           |     |
| +                             | 56             | 42       | 14       | 1.335     | 0.248|
| -                             | 17             | 15       | 2        |           |     |
| bilateral involvement         |                |          |          |           |     |
| bilateral                     | 27             | 20       | 7        | 0.402     | 0.526|
| unilateral                    | 46             | 37       | 9        |           |     |
| vascular tumor thrombus       |                |          |          |           |     |
| +                             | 9              | 6        | 3        | 0.782     | 0.377|
| -                             | 64             | 51       | 13       |           |     |
| capsule invasion              |                |          |          |           |     |
| +                             | 49             | 36       | 13       | 1.853     | 0.173|
| -                             | 24             | 21       | 3        |           |     |
| tumor maximum diameter        |                |          |          |           |     |
| ≤1                            | 34             | 26       | 8        | 0.097     | 0.756|
| ≥1                            | 39             | 31       | 8        |           |     |
| number of foci                |                |          |          |           |     |
| 1                             | 31             | 22       | 9        | 1.594     | 0.207|
| ≥2                            | 42             | 35       | 7        |           |     |
| group                         |                |          |          |           |     |
| ID-CVPTC                      | 25             | 23       | 2        | 3.340     | 0.068|
| MP-CVPTC                      | 17             | 12       | 5        |           |     |
| Background of HT | | | | | |
| + | 18 | 13 | 13 | 72.2% | 5 | 0.479 | 0.489 |
| - | 55 | 44 | 44 | 80.0% | 11 | |

| Psammoma bodies | | | | | |
| + | 24 | 17 | 17 | 10.5% | 7 | 1.098 | 0.295 |
| - | 49 | 40 | 40 | 12.5% | 9 | |

| Nerve invasion | | | | | |
| + | 9 | 7 | 7 | 77.8% | 2 | 0.001 | 0.981 |
| - | 64 | 50 | 50 | 78.1% | 14 | |

| Intraglandular dissemination | | | | | |
| ID-CVPTC | 25 | 23 | 23 | 92.0% | 2 | 4.303 | 0.038 |
| Non ID-CVPTC | 48 | 34 | 34 | 70.8% | 14 | |

**Table 3** Relationship between LVD, MVD and PTC clinicopathological features
| Group               | Total cases | LVD value | MVD value | T value | P value |
|---------------------|-------------|-----------|-----------|---------|---------|
| ID-CVPTC            | 25          | 9.74±2.98 | 9.39±4.33 | 0.680   | 0.499   |
| non ID-CVPTC        | 48          | 7.46±2.55 | 8.62±4.37 |         |         |
| **Age**             |             |           |           |         |         |
| ≤40                 | 56          | 8.95±3.14 | 9.18±4.84 | 0.776   | 0.441   |
| >40                 | 17          | 6.79±1.54 | 8.28±4.34 |         |         |
| **capsule invasion**|             |           |           |         |         |
| +                   | 49          | 8.84±3.04 | 9.45±3.94 | 1.517   | 0.134   |
| -                   | 24          | 7.00±2.13 | 7.73±5.64 |         |         |
| **number of foci**  |             |           |           |         |         |
| 1                   | 31          | 7.50±2.75 | 9.45±5.25 | 0.896   | 0.373   |
| ≥2                  | 42          | 8.79±2.91 | 8.47±4.08 |         |         |
| **tumor maximum diameter** |       |           |           |         |         |
| ≤1                  | 34          | 7.58±3.30 | 8.28±4.84 | 1.040   | 0.302   |
| >1                  | 39          | 8.81±2.68 | 9.41±4.62 |         |         |
| **LNM**             |             |           |           |         |         |
| +                   | 56          | 8.54±2.71 | 8.85±4.66 | 0.096   | 0.924   |
| -                   | 17          | 7.24±3.33 | 8.98±4.54 |         |         |
| **gender**          |             |           |           |         |         |
| man                 | 25          | 8.30±2.46 | 9.45±4.01 | 0.756   | 0.452   |
| female              | 48          | 8.21±3.12 | 8.59±4.90 |         |         |
| **bilateral involvement** |       |           |           |         |         |
| bilateral           | 27          | 8.12±2.54 | 8.27±3.85 | 0.874   | 0.385   |
| unilateral          | 46          | 8.31±3.11 | 9.24±5.00 |         |         |
| **vascular tumor thrombus,** |       |           |           |         |         |
| +                   | 8           | 9.38±2.68 | 10.85±3.14 | 1.287   | 0.202   |
| -                   | 64          | 8.12±2.93 | 8.54±4.71 |         |         |
| **nerve invasion**  |             |           |           |         |         |
| +                   | 8           | 8.10±2.27 | 8.58±3.12 | 0.199   | 0.843   |
|       | 65   | 8.26±2.98 | 8.92±4.77 |
|-------|------|-----------|-----------|
| BRAF-V600E |      |           |           |
| ++    | 57   | 8.32±3.06 | 0.449     | 9.23±4.75 | 1.216 | 0.228 |
| --    | 16   | 7.95±2.28 |           | 7.65±3.94 |       |       |

**Table 4** Relationship between intralobular dissemination and clinicopathological parameter
| Variables                          | Total cases | ID-CVPTC | Non ID-CVPTC | $\chi^2$ | P     |
|-----------------------------------|-------------|----------|--------------|---------|-------|
| **gender**                        |             |          |              |         |       |
| man                               | 25          | 10 (40.0%) | 15           | 0.559   | 0.445 |
| female                            | 48          | 15 (31.3%) | 33           |         |       |
| **LNM**                           |             |          |              |         |       |
| +                                 | 56          | 24 (42.8%) | 32           | 7.918   | 0.005 |
| -                                 | 17          | 1 (5.9%)   | 16           |         |       |
| **bilateral involvement**         |             |          |              |         |       |
| bilateral                         | 27          | 11 (64.7%) | 16           | 0.802   | 0.370 |
| unilateral                        | 46          | 14 (30.4%) | 32           |         |       |
| **vascular tumor thrombus**       |             |          |              |         |       |
| +                                 | 9           | 8 (88.9%)  | 1            | 13.612  | 0.000 |
| -                                 | 64          | 17 (26.6%) | 47           |         |       |
| **capsule invasion**              |             |          |              |         |       |
| +                                 | 49          | 22 (44.9%) | 27           | 7.509   | 0.006 |
| -                                 | 24          | 3 (12.5%)  | 21           |         |       |
| **tumor maximum diameter**        |             |          |              |         |       |
| $\leq 1$                          | 34          | 8 (23.5%)  | 26           | 3.246   | 0.072 |
| $>1$                              | 39          | 17 (43.6%) | 22           |         |       |
| **HT**                            |             |          |              |         |       |
| +                                 | 18          | 5 (27.8%)  | 13           | 0.444   | 0.505 |
| -                                 | 55          | 20 (36.4%) | 35           |         |       |
| **psammoma bodies**               |             |          |              |         |       |
| +                                 | 24          | 13 (54.2%) | 11           | 6.301   | 0.012 |
| -                                 | 49          | 12 (24.5%) | 37           |         |       |
| **nerve invasion**                |             |          |              |         |       |
| +                                 | 9           | 5 (55.6%)  | 4            | 2.070   | 0.038 |
| -                                 | 64          | 20 (31.3%) | 44           |         |       |
Figures

Figure 1

Technical route
Figure 2

Morphological characteristics of HE (lower right corner shows images obtained under a high power microscope): A. CVPTC with a papillary branching structure (50×), and classic CVPTC nuclei in tumor cells under a high power microscope (400×); B. ID-CVPTC showing an unclear tumor boundary (50×) and a tumor nest interspersed within surrounding thyroid tissue (400×); C. The surrounding infiltrating foci were glandular cavities or masses (200×); cytosolic eosinophilia of tumor cells (400×); D. Noncontinuous independent infiltrating foci were found in the thyroid tissue far away from the largest tumor (> 0.5 cm), and there was no interstitial reaction between the tumor and the surrounding thyroid (× 200); the tumor showed ground glass-like nuclei and abundant cytoplasm (400×).
Figure 3

Positive expression of BRAF-V600E: A. ID-CVPTC HE staining (× 200); B. Immunohistochemical staining. BRAF-V600E was mainly localized in the cytoplasm of ID-CVPTC, while no expression was detected in adjacent nontumor tissues (× 200); C. SF-CVPTC HE staining (× 200); D. BRAF V600E showed positive expression in the cytoplasm of SF-CVPTC tumor cells but not in the adjacent nontumor tissues (× 200).
Figure 4

LVD and MVD determination (200×): A. loss of lymphatic vessels in the tumor; B. dense blood vessels in the tumor; C-D. The vascular density of the tumor margin was high; E-F. The vascular density of normal thyroid tissue around the tumor was low.
Figure 5

Disseminated tumor thrombus in lymphatic vessels in the PTC glandular lobe (200×): A. HE: The tumor thrombus and psammoma bodies were found on the edge of the tumor; B. D2-40 staining showing lymphatic vessels; C. CD31 staining showing a tumor thrombus with abundant blood vessels among tumor cells; D. The positive expression of BRAF-V600E protein was consistent with that of PTC.