Value of biomarkers in distinguishing indeterminate core needle biopsy samples of thyroid nodules

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Abstract

Background: Core needle biopsy (CNB) is now more frequently used for the preoperative diagnosis of thyroid nodules. Based on morphology alone, 5%-20% of CNB samples cannot be determined as malignant or benign. Compared to fine-needle biopsy (FNB), samples collected by CNB are more accessible for various tests. Therefore, studying the application of biomarkers in distinguishing indeterminate CNB samples of thyroid nodules is a practical need.

Methods: Patients with thyroid nodules with both CNB and matched resected specimens were reviewed. Cases classified as indeterminate lesions, follicular neoplasms and suspicious for malignancy were retrieved. All CNB samples were stained by immunohistochemistry (IHC) using antibodies against CK19, Galectin-3, HBME-1, and CD56 and detected by next-generation sequencing (NGS) using a target panel. With the help of these biomarkers, all CNB samples were reclassified. Taking the classification of resected specimens as the gold standard, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of each biomarker for discriminating malignancy from benignity were calculated.

Results: The sensitivity, specificity, PPV, NPV and accuracy were 93.55%, 60.00%, 93.55%, 60.00% and 88.89% for CK19; 93.55%, 40.00%, 90.63%, 50.00% and 86.11% for Galectin-3; 77.42%, 100.00%, 100.00%, 41.67% and 80.56% for HBME-1; 66.13%, 100.00%, 100.00%, 32.26% and 70.83% for CD56; and 91.94%, 100.00%, 100.00%, 66.67% and 93.06% for pathogenic mutation.

Conclusions: The application of biomarkers is very effective in distinguishing indeterminate CNB samples of thyroid nodules. Gene testing by NGS using a target panel has very high accuracy. The limitation of tumor quantity is the main reason for the weakened power of NGS. IHC plays an important role in cases with negative NGS results. The combination of NGS and IHC is a reliable “rule in” test for malignancy.

Background

Thyroid nodules are a common disease of the endocrine system. The prevalence is 20–76% in the Chinese population as identified by high-resolution ultrasound, and 5–15% of nodules are malignant [1]. It is very important to screen these malignant cases for further treatment.

Core needle biopsy (CNB) was first used in the preoperative diagnosis of thyroid nodules in the 1990s [2]. CNB has not been widely used for many years because of the pain, tolerability and complications associated with the operation. However, with advances in CNB devices and the development of high-resolution ultrasound, these disadvantages have been significantly reduced. Recently, several large single-center studies have shown no significant differences between fine-needle biopsy (FNB) and CNB in terms of pain, tolerability, or complications [3, 4]. In this case, the advantage of CNB for obtaining a large amount of tissue and providing more information on histological structures is highlighted. Published studies have shown that the accuracy of CNB for the preoperative diagnosis of thyroid nodules was higher than that of FNB [5]. However, approximately 5%-20% of CNB samples are classified as

indeterminate based on morphology alone [5, 6]. Compared to FNB, samples collected by CNB are more accessible for various testing methods. Therefore, studying the application of biomarkers in distinguishing indeterminate CNB samples of thyroid nodules is a practical need. We retrieved 72 cases of indeterminate CNB of thyroid nodules that had matched resected specimens. Taking the diagnoses of the matched resected specimens as the gold standard, we studied the capability of biomarkers in distinguishing indeterminate samples as either malignant or benign.

Methods

Patients and samples

Patients with thyroid nodules with both CNB and matched resected specimens treated at Peking University First Hospital between January 2015 and December 2017 were reviewed. CNB was used as the first-line preoperative diagnosis in all cases without prior FNB and was performed according to the recommendations from publications [7].

According to the proposal of the Korean Thyroid Association, the diagnoses of CNB specimens were divided into the following six categories: I. unsatisfactory; II. benign; III. indeterminate lesion; IV, follicular neoplasm; V, suspicious for malignancy; and VI, malignant [8]. Cases classified as III-V were retrieved.

Pathological Review

All hematoxylin and eosin (H&E) staining slides were separately reviewed by two pathologists blinded to the original diagnoses. The CNB samples were diagnosed according to the Korean proposal (Table 1) [8]. The resected samples were diagnosed according to the 2017 WHO classification of tumors of endocrine organs (4th)[9]. Cases with inconsistent diagnoses were reviewed, and agreements were achieved by discussion.

Table 1

Diagnostic Categories of Thyroid Core Needle Biopsy Proposed by the Korean Thyroid Association

I. Nondiagnostic or unsatisfactory
• Normal thyroid tissue only
• Extrathyroid tissue only (e.g., skeletal muscle, mature adipose tissue)
• A virtually acellular specimen
• Acellular/paucicellular fibrotic nodule
• Blood clot only
• Other
II. Benign lesion
• Benign follicular nodule or consistent with a benign follicular nodule
• Hashimoto's thyroiditis
• Granulomatous (subacute) thyroiditis
• Nonthyroidal lesion (e.g., parathyroid lesions, benign neurogenic tumors, benign lymph node)
• Other
III. Indeterminate lesion
IIIA. Indeterminate follicular lesion with nuclear atypia
• Follicular proliferative lesions with focal nuclear atypia
• Follicular proliferative lesions with equivocal or questionable nuclear atypia
• Atypical follicular cells embedded in a fibrotic stroma
IIIB. Indeterminate follicular lesion with architectural atypia
• Microfollicular proliferative lesion lacking a fibrous capsule or the adjacent nonlesional tissue in the specimen
• Solid or trabecular follicular lesion lacking a fibrous capsule or the adjacent nonlesional tissue in the specimen
• Macrofollicular proliferative lesion with a fibrous capsule
• Hürthle cell proliferative lesion lacking a fibrous capsule or the adjacent nonlesional tissue in the specimen
IIIC. Other indeterminate lesions
IV. Follicular neoplasm or suspicious for a follicular neoplasm
• Microfollicular proliferative lesion with a fibrous capsule

I. Nondiagnostic or unsatisfactory
• Mixed microfollicular and normofollicular proliferative lesion with a fibrous capsule
• Solid/trabecular follicular proliferative lesion with a fibrous capsule
• Hürthle cell proliferative lesion with a fibrous capsule
• Follicular neoplasm with focal nuclear atypia
V. Suspicious for malignancy
• Suspicious for papillary carcinoma, medullary carcinoma, poorly differentiated carcinoma, metastatic carcinoma, lymphoma, etc.
VI. Malignant
• Papillary thyroid carcinoma, poorly differentiated carcinoma, undifferentiated (anaplastic carcinoma), medullary thyroid carcinoma, lymphoma, metastatic carcinoma, etc.

Immunohistochemistry

The primary antibodies included antibodies against CK19 (Dako, Clone RCK108), Galectin-3 (Invitrogen, A3A12), HBME-1 (Dako, Clone HBME-1) and CD56 (Dako, Clone 123C3). The antigen retrieval buffer was EDTA (pH 9.0), the temperature was 98 °C and the duration was 20 minutes. We used EnVision FLEX + Mouse LINKER to amplify the signal and the EnVision FLEX Mini Kit to visualize the IHC reaction together with Autostainer Link 48 (Agilent Technologies, Santa Clara, CA, United states).

Evaluation of immunohistochemical staining

Tumors with cytoplasmic ± membranous reactivity for CK19 in more than 10% of cells with strong intensity were considered positive. Tumors with cytoplasmic + nuclear reactivity for Galectin-3 and membranous reactivity for HBME-1 or CD56 in more than 10% of cells were considered positive without regard to intensity [10].

Next-generation sequencing

The percentage of tumor components in the whole sample of CNB specimens was recorded. Genomic DNA was extracted from unstained 5-μm-thick paraffin embedded sections using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. After extraction, DNA quality was evaluated by 1% agarose gel electrophoresis. The concentration of all samples was quantitated by a NanoDrop system (Invitrogen Life Technologies, Carlsbad, CA, USA) and Qubit Fluorometer (Invitrogen Life Technologies).

Targeted next-generation sequencing (NGS) was conducted using an OncoAim® thyroid cancer multigene assay kit (Singlera Genomics, Inc., Shanghai, China) that detected 26 genes (Table 2). According to the kit protocol, 50 ng of DNA for each sample was used to generate sequencing libraries. DNA was fragmented by 5X WGS Fragmentation Mix (Qiagen, Beverly, MA, USA). After quality control and

quantification, the library product was sequenced using 150 bp paired-end runs on the NextSeq 500 platform (Illumina, Inc., San Diego, CA, USA). Sequencing data were then aligned to the reference Human Genome (hg19). Read mapping, quality control, variant calling, and genotyping were performed automatically using the Tools Kit supplied in the OncoAim® Kit (Singlera), and the minimum confidence threshold for variant calling was set to 5%. Variant functional annotation was performed with the ENSEMBL Variant Effect Predictor tool.

Table 2

Genes Detected by OncoAim® Thyroid Cancer Multigene Assay Kit

Gene	Transcript	variation type	
		Mutation	Fusion
BRAF	NM_004333	exon 15	intron 7–10
RET	NM_020975	exon 7–16	intron 10–11
NRAS	NM_002524	exon 2–3	-
KRAS	NM_033360	exon 2–4	-
HRAS	NM_176795	exon 2–3	-
AKT1	NM_005163	exon 2–7, exon 9–12	-
ATM	NM_000051	all exon	-
CNNB1	NM_001904	all exon	-
TSHR	NM_000369	all exon	-
APC	NM_000038	all exon	-
TTN	NM_001256850	all exon	-
TG	NM_003235	all exon	-
RB1	NM_000321	all exon	-
MEN1	NM_000244	all exon	-
PDGFRA	NM_006206	all exon	-
PIK3CA	NM_006218	all exon	-
CDKN2A	NM_000077	all exon	-
EIF1AX	NM_001412	all exon	-
PTEN	NM_000314	exon 5–8	-
GNAS	NM_000516	exon 8–9	-
TP53	NM_000546	exon 5–9	-
TERT	*NM_198253*	Promoter (chr5:1295183–1295302)	-
PPARG	NM_005037	-	intron 1
NTRK1	NM_002529	-	intron 9, exon 12

Gene	Transcript	variation type	
		Mutation	Fusion
NTRK3	NM_002530	-	intron 13
ALK	NM_004304	-	intron 16, intron 19

Based on ClinVar (Version 20280919), the result was marked as pathogenic, likely pathogenic, uncertain significance, likely benign, benign or inconclusive. The sample was recorded as positive for NGS in cases with a confirmed pathogenic or likely pathogenic mutation status.

Reclassification of CNB samples with the help of biomarkers

According to the rule of CK19 positive, Galectin-3 positive, HBME-1 positive, CD56 negative and NGS positive implying malignancy, we reclassified all CNB samples, and the result was named CNB A, B, C, D and E with IHC of CK19, Galectin-3, HBME-1, CD56 and NGS, respectively.

Statistical Analysis

Taking the diagnosis of the resected specimens as the gold standard, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of each biomarker for discriminating malignancy from benignity were calculated. The formulas were as follows.

Sensitivity = Number of cases classified as “malignant” based on both the CNB and resected specimens/Number of cases classified as “malignant” based on both the CNB and resected specimens + Number of cases classified as “benign” based on the CNB specimen but as “malignant” based on the resected specimen × 100%.

Specificity = Number of cases classified as “benign” based on both the CNB and resected specimens/Number of cases classified as “benign” based on both the CNB and resected specimens + Number of cases classified as “malignant” based on the CNB specimen but as “benign” based on the resected specimen × 100%.

PPV = Number of cases classified as “malignant” based on both the CNB and resected specimens/Number of cases classified as “malignant” based on both the CNB and resected specimens + Number of cases classified as “malignant” based on the CNB specimen but as “benign” based on the resected specimen × 100%.

NPV = Number of cases classified as “benign” based on both the CNB and resected specimens/Number of cases classified as “benign” based on both the CNB and resected specimens + Number of cases classified as “benign” based on the CNB specimen but as “malignancy” based on the resected specimen × 100%.

Accuracy = Number of cases classified as “malignant” based on both the CNB and resected specimens + Number of cases classified as “benign” based on both the CNB and resected specimens/Number of all cases × 100%.

Results

Patients

The study included 72 patients. Of them, 19 were males and 53 were females, with ages ranging from 20 to 81 years and a mean age of 49 years old.

Histological morphology

The morphology of all CNB samples was entirely follicular architecture with a nuclear score of 1–2 [9]. The diagnoses of the resected specimens included 5 cases of nodular hyperplasia, 4 cases of thyroiditis, 1 case of follicular adenoma (FA), 1 case of follicular thyroid carcinoma (FTC), 26 cases of follicular variant of papillary thyroid carcinoma (FVPTC) and 35 cases of conventional papillary thyroid carcinoma (CPTC) with a follicular predominant growth pattern (Fig. 1, 2).

Results of IHC

Of 72 cases, 62 cases (86.1%) were positive for CK19, 64 cases (88.9%) were positive for Galectin-3, 48 cases (66.7%) were positive for HBME-1, and 41 cases (56.9%) were negative for CD56 (Fig. 1, 2).

Results of NGS

Of 72 cases, 57 cases (79.17%) were positive for NGS, including 47 cases of BRAFV600E, 4 cases of RET fusion, 2 cases of NTRK fusion, 2 cases of KRAS mutation and 2 cases of TSHR mutation.

Reclassification of CNB samples with the help of biomarkers

CNB A included 10 benign and 62 malignant cases with CK19 IHC. CNB B included 8 benign and 64 malignant cases with Galectin-3 IHC. CNB C included 24 benign and 48 malignant cases with HBME-1 IHC. CNB D included 31 benign and 41 malignant cases with CD56 IHC. CNB E included 15 benign and 57 malignant cases with NGS. The agreement with the classification of resected specimens is shown in Table 3.

Table 3
Comparison Between Reclassification of CNB Samples with the help of Biomarkers and Classification of Matched Resected Specimens

		Matched Resected Specimens, No.		
	CNB Samples, No.	Benignity	Malignancy	Total
CNB A	Benignity	6	4	10
	Malignancy	4	58	62
CNB B	Benignity	4	4	8
	Malignancy	6	58	64
CNB C	Benignity	10	14	24
	Malignancy	0	48	48
CNB D	Benignity	10	21	31
	Malignancy	0	41	41
CNB E	Benignity	10	5	15
	Malignancy	0	57	57
	Total	10	62	72

CNB, core needle biopsy

Predictive value of CNB with the help of biomarkers

Taking the classification of the resected specimens as the gold standard, the sensitivity, specificity, PPV, NPV and accuracy were 93.55%, 60.00%, 93.55%, 60.00% and 88.89% for CNB A; 93.55%, 40.00%, 90.63%, 50.00% and 86.11% for CNB B; 77.42%, 100.00%, 100.00%, 41.67% and 80.56% for CNB C; 66.13%, 100.00%, 100.00%, 32.26% and 70.83% for CNB D; and 91.94%, 100.00%, 100.00%, 66.67% and 93.06% for CNB E (Table 4).

Table 4
Predictive Value of CNB with the Help of Biomarkers

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
CNB A	93.55	60.00	93.55	60.00	88.89
CNB B	93.55	40.00	90.63	50.00	86.11
CNB C	77.42	100.00	100.00	41.67	80.56
CNB D	66.13	100.00	100.00	32.26	70.83
CNB E	91.94	100.00	100.00	66.67	93.06
CNB, core needle biopsy					
PPV, positive predictive value; NPV, negative predictive value					

Discussion

Morphological changes, including nuclear score, architecture (papillary or follicular), and growth pattern (infiltrative or encapsulated), are the key points for diagnosing thyroid tumors. Based on the criteria above, major cases can be diagnosed undoubtedly. However, some cases are difficult to determine based on histological morphology alone. Compared to resected specimens, the diagnoses of biopsies are more challenging. The indeterminate rate of diagnosis is reported to be 10%-40% for FNB and 5%-20% for CNB [5]. Our comparative study between CNB and resected specimens of thyroid nodules showed that 74 of 578 cases were unable to be determined as malignant or benign based on CNB sample morphology alone [6]. The reason is that only follicles visible on CNB in addition to the presence of atypical nuclei and the absence of normal tissue as background makes it impossible to differentiate FTC, FVPTC, and CPTC with a follicular predominant growth pattern from FA, nodular hyperplasia and thyroiditis. Therefore, studying the application of biomarkers in distinguishing indeterminate biopsy samples is necessary.

IHC is the most popular ancillary technique used in pathological practice. Studies on resected specimens showed that CK19, Galectin-3, HBME-1 and CD56 were very helpful in discriminating malignancy from benignity [10–13]. In Dunderovic et al.'s study, the sensitivity of CK19, Galectin-3, HBME-1, and CD56 was 75.41%, 88.52%, 71.31%, and 58.20%, respectively, and the specificity of CK19, Galectin-3, HBME-1, and CD56 was 70.89%, 64.56%, 84.81%, and 92.41% [10]. Our experience in clinical practice is similar to the results. Based on the knowledge above, it was supposed that IHC may play a role in improving the accuracy of diagnosing indeterminate biopsy samples. We searched papers published in English in PubMed and found only one focusing on this topic. In this paper, Song et al. reported that the continued indeterminate rate was 42.9% for FNB and 11.3% for CNB after IHC was applied [14]. In our study, all 72 indeterminate samples of CNB could be determined with the help of IHC, although the accuracy of each marker was different. Taking the diagnosis of the resected specimens as the gold standard, HBME-1 and CD56 are extremely specific, with a specificity of 100% and a PPV of 100%. CK19 is the most sensitive,

with a sensitivity of 93.55% and an NPV of 60.00%. Galectin-3 was less optimal, with a sensitivity of 93.55% and an NPV of 50.00%. The overexpression of HBME-1 or loss of CD56 expression strongly suggested malignancy. However, negative HBME-1 and/or positive CD56 should be cautiously considered as a sign of benignity, especially in those with overexpression of CK19 or Galectin-3 at the same time. Samples negative for HBME-1 and/or positive for CD56 as well as overexpression of CK19 or Galectin-3 should be recommended for rebiopsy, given that nodules treated with CNB are usually suspected of malignancy by ultrasound.

In the past 10 years, we have witnessed significant progress in the field of the molecular pathogenesis of thyroid carcinoma based on studies using NGS. In 2014, The Cancer Genome Atlas (TCGA) reported the comprehensive genomic characteristics of PTC. Ninety-seven percent of PTCs have unique molecular alterations, of which BRAF V600E mutations, RAS mutations, RET fusions, and TERT mutations are common, and EIF1AX mutations, ALK fusions, and NTRK1 or NTRK3 fusions are uncommon [15]. Subsequently, the genotypes of FTC, poorly differentiated thyroid carcinoma (PDTC) and anaplastic thyroid carcinoma (ATC) have also been reported. Of the molecular alterations of FTC, RAS mutations, PAX8-PPARγ fusions and TERT mutations are common, and TSHR mutations, BRAF K601E mutations and EIF1AX mutations are uncommon. Of the molecular alterations of PDTC as well as ATC, BRAF V600E mutations, RAS mutations, TERT mutations and TP53 mutations are common [16–18]. Based on their own understanding of the mutational profile of thyroid carcinoma, researchers have tried to use diverse molecular approaches to improve the accuracy of diagnosing indeterminate biopsy samples and have presented various published results. The sensitivity and specificity of gene testing for discriminating malignancy from benignity were 63%-94% and 52%-99%, respectively, with FNB [19–21]. Regardless of how sensitive or specific it is, applying gene testing to FNB is limited in clinical practice because specialized sample collection is required at the time of the initial procedure. In contrast, CNB samples are routinely stored as paraffin-embedded blocks in which DNA can readily be extracted at any moment. In this case, gene testing is supposed to be used in distinguishing indeterminate CNB samples more practically and effectively than FNB. Compared to the numbers of studies about FNB, papers published about CNB are very limited. To date, only a few single mutations have been reported [22–25]. In this study, we detected indeterminate CNB samples by NGS using a target panel that covered the major molecular alterations of thyroid carcinoma. The sample was recorded as positive for NGS in cases of confirmed pathogenic or likely pathogenic mutations. Taking the diagnosis of the resected specimens as the gold standard, NGS is extremely specific and sensitive, with a specificity of 100%, a PPV of 100%, a sensitivity of 91.94% and an NPV of 66.67%. Considering both sensitivity and specificity, gene testing by NGS using a target panel was the most effective, with an accuracy of 93.06%. Our study shows that the application of NGS using a target panel for distinguishing indeterminate CNB samples is not only available but also very capable. In our study, all 10 cases classified as benign on resected specimens were negative for NGS on CNB, and of the 62 cases classified as malignant on resected specimens, only 5 cases were negative for NGS on CNB. Of the five, 4 cases with tumor components less than 5% were detected to have BRAF V600E mutations on resected specimens, and 1 case with tumor components of 10% was confirmed to be negative for NGS on resected specimens. Therefore, the limitation of tumor

quantity is still the main reason for the weakened power of NGS in distinguishing indeterminate samples of CNB, even though the influence is lower than that of FNB. We reviewed these five cases and found that all of them were positive for CK19, Galectin-3, and HBME-1 and negative for CD56 on CNB. These results showed that IHC plays an important role in cases with negative NGS results.

Conclusions

The application of biomarkers is very effective in distinguishing indeterminate CNB samples of thyroid nodules. The overexpression of HBME-1, loss of CD56 expression or presence of pathogenic mutations are the most specific. The overexpression of CK19 or Galectin-3 is the most sensitive. Given both sensitivity and specificity, gene testing by NGS using a target panel has the highest accuracy of 93.06%. The limitation of tumor quantity is the main reason for the weakened power of NGS. IHC plays an important role in cases with negative NGS results. The combination of NGS and IHC is a reliable “rule in” test for malignancy. The limitation of this study is that only 72 cases were retrieved, and 61 of 62 malignant cases were PTC. Therefore, the conclusions should be tested in more cases with diverse types of thyroid carcinoma by more studies.

Abbreviations

CNB: Core needle biopsy; FNB: Fine needle biopsy; H&E: hematoxylin and eosin; IHC: immunohistochemistry; NGS: next-generation sequencing; PPV: Positive predictive value; NPV: Negative predictive value; FA: follicular adenoma; FTC: follicular thyroid carcinoma; PTC: papillary thyroid carcinoma; FVPTC: follicular variant of papillary thyroid carcinoma; CPTC: conventional papillary thyroid carcinoma; TCGA: The Cancer Genome Atlas; PDTC : poorly differentiated thyroid carcinoma; ATC: anaplastic thyroid carcinoma

Declarations

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Availability of data and material

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

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Author's contributions

YX initiated the study and wrote the manuscript. LL did administration of the project and checked the results of gene testing. LY collected data and reviewed slides. DL did IHC testing. XL did gene testing. JD collected and managed data. YZ checked the results of gene testing. SH reviewed slides. TL supervised the research. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All patient samples and clinical data using were approved by the Ethics committee of the Peking University First Hospital and the exemption from informed consent was approved as well [Ethical approval No.: (2018) Research No. 147].

Consent for publication

Not applicable

Competing interests

None declared

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Figures

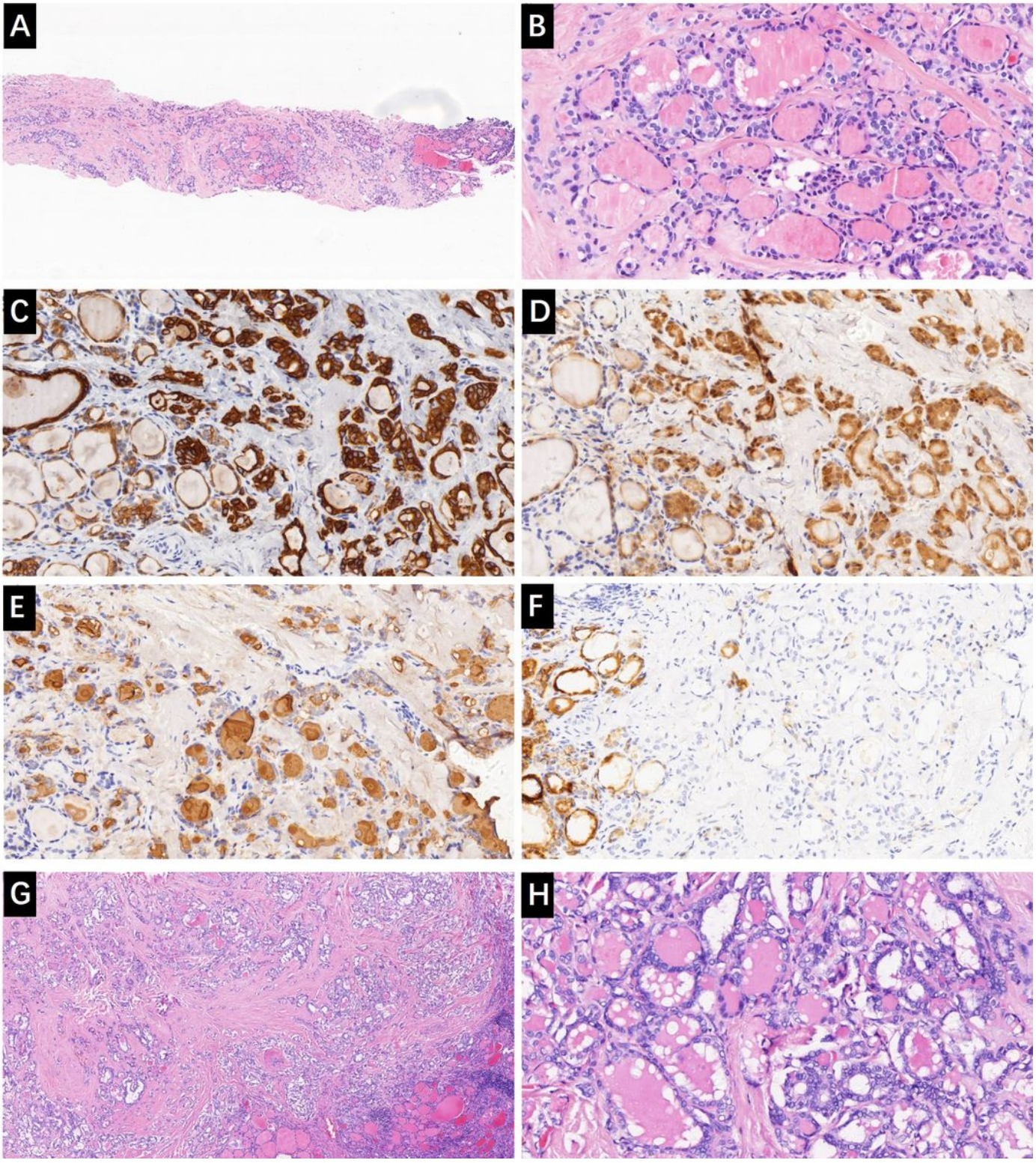

Figure 1

Case classified as indeterminate lesion in the CNB sample while follicular variant of papillary thyroid carcinoma in the matched resected specimen. A, Tumor in the CNB sample is entirely composed of follicular structures (H&E×40). B, High magnification of the lesion shows the follicular structures lined by cells with nuclei scored 1 (H&E×200). C, Cytoplasm and membrane of tumor cells in the CNB sample are diffusely reactive for CK19 with strong intensity, while the normal follicular cells are reactive with weak

intensity (CK19×200). D, Cytoplasm and nuclei of tumor cells in the CNB sample are diffusely reactive for Galectin-3 with strong intensity, while the normal follicular cells are nonreactive (Galectin-3×200). E, Membrane of tumor cells in the CNB sample are partially (about 30%) reactive for HBME-1 with intermediate intensity, while the normal follicular cells are nonreactive (HBME-1×200). F, Tumor cells in the CNB samples are nonreactive for CD56, while membrane and cytoplasm of the normal follicular cells are diffusely reactive with strong intensity (CD56×200). G, Tumor in the matched resected specimen is entirely composed of follicular structures. (H&E×40). H, High magnification of the lesion shows the follicular structures lined by cells with nuclei scored 3 (H&E×200).

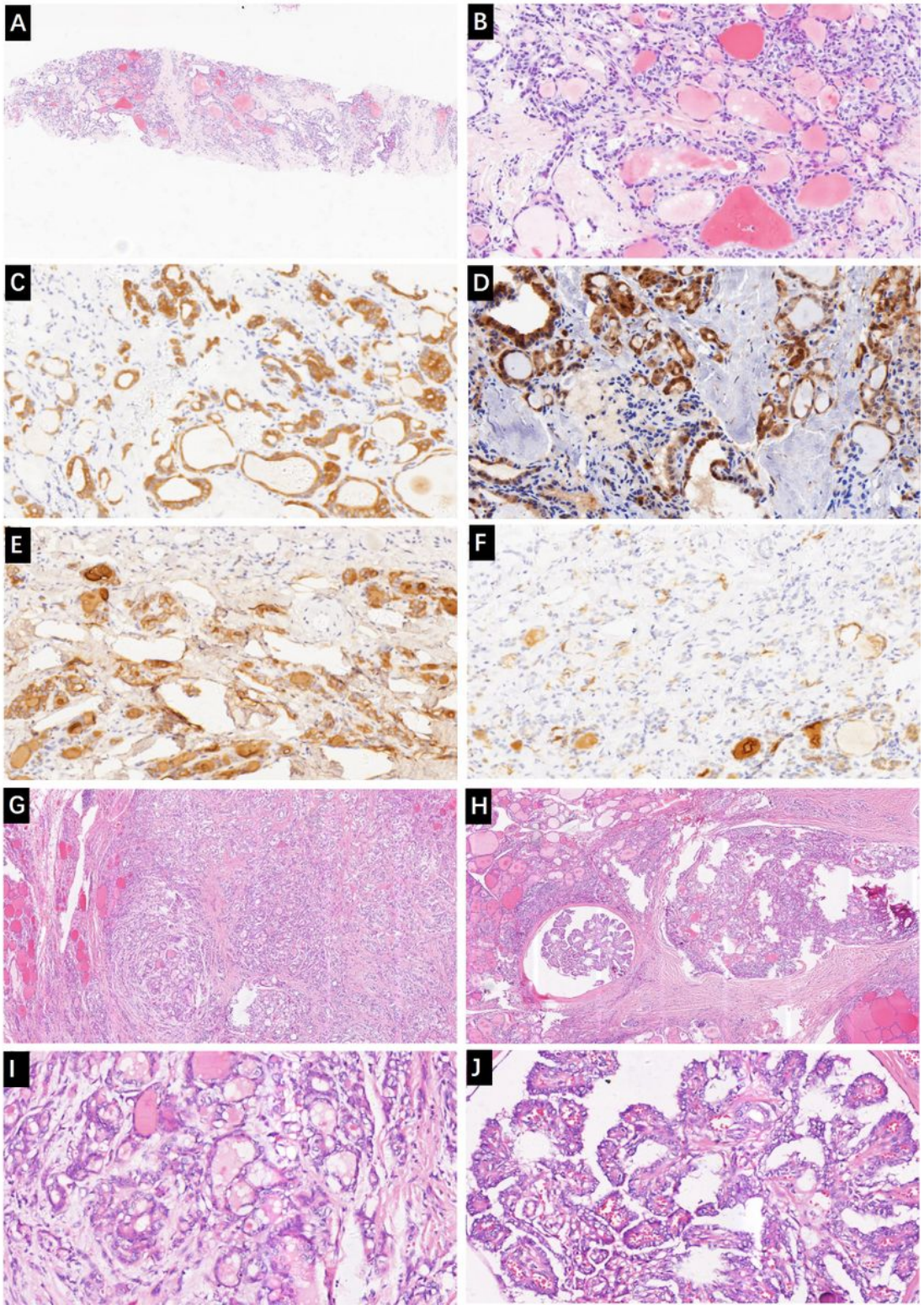

Figure 2

Case classified as indeterminate lesion in the CNB sample while conventional papillary thyroid carcinoma with a follicular predominant growth pattern in the matched resected specimen. A, Tumor in the CNB sample is entirely composed of follicular structures (H&E×40). B, High magnification of the lesion shows the follicular structures lined by cells with nuclei scored 2 (H&E×200). C, Cytoplasm and membrane of tumor cells in the CNB sample are diffusely reactive for CK19 with strong intensity, while the normal

follicular cells are nonreactive (CK19×200). D, Cytoplasm and nuclei of tumor cells in the CNB sample are diffusely reactive for Galectin-3 with strong intensity, while the normal follicular cells are nonreactive (Galectin-3×200). E, Membrane of tumor cells in the CNB sample are diffusely reactive for HBME-1 with strong intensity, while the normal follicular cells are nonreactive (HBME-1×200). F, Tumor cells in the CNB samples are nonreactive for CD56, while membrane and cytoplasm of the normal follicular cells are diffusely reactive with intermediate intensity (CD56×200). G, H, Tumor in the matched resected specimen is almost entirely composed of follicular structures, except of focal papillary structure (H&E×40). I, High magnification of the lesion shows the follicular structures lined by cells with nuclei scored 3 (H&E×200). J, High magnification of the lesion shows the papillary structures lined by cells with nuclei scored 3 (H&E×200).