

Total thyroidectomy can still remain the method of choice in some Bethesda III cases

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Background. The latest WHO classification of tumours of endocrine organs defines new units of borderline thyroid tumours (BTT). The aim of our study was to evaluate ultrasonographic and cytological features, mutation profile and surgery treatment in rare thyroid tumours.

Methods. An analysis of 8 BTT out of 487 patients, who underwent thyroid surgery between June 2016 and June 2020. The definitive diagnosis was made postoperatively by extensive histopathological examination. Molecular genetic analysis of genes associated with thyroid oncology (*BRAF*, *HRAS*, *KRAS*, *NRAS*, *TERT*, *TP53*, fused genes) were performed from one FNAB, and 7 formalin-fixed paraffin-embedded (FFPE) samples.

Results. BTT were found in a total of 8 patients (1.6%), with a predominance of men with respect to other operated patients. FNAB samples were classified in the Bethesda system as Bethesda I, Bethesda II and Bethesda III in one, four and three cases, respectively. Hemithyroidectomy and total thyroidectomy were performed equally in four patients. The histopathological diagnosis revealed non-invasive encapsulated follicular neoplasm with papillary-like nuclear features (NIFTP) in three patients, follicular tumour of uncertain malignant potential (FT-UMP) in three patients, well differentiated tumour of uncertain malignant potential (WDT-UMP) in one patient, and hyalinizing trabecular tumour (HTT) in one case. In NIFTP cases mutation in *HRAS* gene in one patient together with probable pathogenic variant in *TP53* gene and in *NRAS* gene in two patients were detected. In HTT patient *PAX8/GLIS3* fusion gene was detected.

Conclusion. The surgical treatment of BTT is necessarily individual influenced by preoperative clinical, ultrasonographic, cytological and molecular genetic findings, and the presence of other comorbidities.

Key words: borderline thyroid tumour, FNAB, molecular testing of thyroid, thyroidectomy

Received: June 10, 2021; Revised: June 10, 2021; Accepted: June 30, 2021; Available online: July 20, 2021

<https://doi.org/10.5507/bp.2021.045>

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INTRODUCTION

The 4th edition of the WHO classification of tumours of the endocrine organs 2017, defined new units of borderline thyroid tumours (BTT) filling a grey area in pathological diagnosis between benign and malignant follicular cell derived tumours. These encompass units of encapsulated or well-circumscribed tumours with uncertain invasion of tumour cells into the tumour capsule or vessels with missing or questionable nuclear features of papillary thyroid carcinoma -follicular tumour of uncertain malignant potential (FT-UMP) and well differentiated tumours of uncertain malignant potential (WDT-UMP), respectively¹. Papillary thyroid carcinoma (PTC) is the most frequent cancer of the thyroid gland and is divided into several histological variants with the follicular variant (FV PTC) as one of the most common. A subset of encapsulated FV PTC (EFVPTC) without evidence of capsular and/or lymphovascular invasion were reclassified as non-invasive

follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) by the Nikiforov group in 2016 (ref.²).

NIFTP can be suggested as a differential diagnostic option in FNAB (ref.³⁻⁵) nevertheless, it is definitively diagnosed, similar to other follicular tumours following extensive histopathological examination. The diagnostic criteria of NIFTP include encapsulation or partial encapsulation with clear demarcation, follicular pattern, no psammoma bodies, absence of invasion, necrosis or higher mitotic activity and less than 30% of the tumour volume showing a trabecular, insular or solid architecture. The nuclear features of papillary carcinoma must be expressed by the WHO newly standardized score of 2 or 3 (ref.¹). The original criteria allowed <1% papillae¹, but current criteria do not allow any well-formed papillary structures. Finding of the *BRAF* V600E mutation or other mutations (e.g. *RET/PTC*) or high-risk mutations (e.g. *TERT* promoter, *TP53*) precludes a diagnosis of NIFTP (ref.⁶).

About 20–30% of samples^{7,8} obtained by fine-needle aspiration biopsy are classified as uncertain/suspected category III-V in the BSRTC (Bethesda System for Reporting Thyroid Cytopathology) classification. In preoperative diagnosis, the determination of genetic markers may be beneficial. With their help, these tumours can be better stratified in terms of aggression and risk of disease recurrence and overtreatment in low-risk tumours can be avoided. Mutation of the RAS gene is considered to be the dominant genetic finding especially in NIFTP. Other mutations e.g. *PAX8/PARG* rearrangement, *THADA* (thyroid adenoma-associated) fusions, *EIF1AX* and occasionally *BRAF* K601 mutation can be helpful to suggest NIFTP preoperatively⁹.

Hyalinizing trabecular tumour (HTT) is a rare encapsulated or well-circumscribed tumour consisting of solid-alveolar to trabecular arrangement of cells, with elongated to round nuclei, noticeable nuclear grooves and intranuclear pseudoinclusions, intertrabecular and pericellular hyaline material. HTT tumour cells show positive expression of TTF-1, thyroglobulin and characteristic membrane positivity of Ki-67 (ref.^{1,10}). A key finding in preoperative diagnosis is the detection of the *PAX8/GLIS3* fusion gene^{11,12} that is considered to be specific for this type of tumour. Data on long-term follow-up of these lesions are limited, however, the incidence of recurrences and metastasis is quite rare in less than 0.2% of cases⁷. Follicular adenoma, adenomatous node, FV PTC, follicular thyroid carcinoma (FTC), and medullary carcinoma (MTC) must be ruled out in the differential postoperative diagnosis^{13,14}. HTT and NIFTP are mostly tumours with indolent behaviour. A conservative approach to surgical treatment (lobectomy) is an effective procedure in these tumours, unless TSH suppression and iodine radiotherapy are required in patients^{13,15}.

The aim of this study was to evaluate preoperative ultrasonographic and cytological features and molecular genetic results and, to comment on the extent and results of operations in individuals with the rare BTT of the thyroid gland. As an illustration, we present a case report of a patient with HTT.

MATERIALS AND METHODS

An analysis of 487 operated patients, 8 of whom had a histopathologically proven rare tumour of uncertain biological behaviour – BTT. They were operated in the range of HTE/TTE by one surgeon (JL) at the Department of ENT and Head and Neck Surgery at Na Homolce Hospital (NNH), in the period 6/2016–6/2020. The postoperative follow-up period was 6–65 months. The operations were performed on the basis of clinical, ultrasonographic (USG) and FNAB findings under USG control. Cytological samples were classified according to BSRTC (2017). Postoperative histopathological diagnosis was determined by two experienced pathologists at the Department of Pathology NNH (MS) and the Institute of Pathology 1st Faculty of Medicine and Faculty General Hospital, Prague (JD). Molecular genetic testing

of one FNAB and 7 formalin-fixed paraffin-embedded (FFPE) was performed in the Department of Molecular Endocrinology of the Institute of Endocrinology in Prague (VS).

DNA and RNA extraction

AllPrep DNA/RNA/miRNA Universal Kit (Qiagen) was used for extraction of DNA and RNA from FNAB and AllPrepDNA/RNA FFPE Kit (Qiagen) for extraction of DNA and RNA from FFPE according to the manufacturer's instructions. DNA and RNA concentrations were measured using a fluorometer (Qubit 2.0, Invitrogen).

Detection of point mutations in FNAB

According to our diagnostic procedure, firstly, FNA samples were analysed for *BRAF* V600E mutation using allele-specific Real Time PCR using *BRAF* Mutation Analysis Kit II (EntroGen). If no *BRAF* V600E mutation was found, the analysis continued by next generation sequencing using Thyro-ID kit (4bases) based on targeted amplification of 14 genes including *HRAS* (exon 2, 3, NM_005343.4), *KRAS* (exon 2, 3, NM_033360.4), *NRAS* (exon 2, 3, NM_002524.5), *BRAF* (exon 15, NM_004333.6) and *TERT* (promoter, NM_198253.3) genes and sequencing on Miseq (Illumina). Bioinformatic data evaluation was performed using VarSome Clinical (Saphetor SA).

Detection of *BRAF*, *HRAS*, *KRAS* and *NRAS* mutations in FFPE samples

The detection of point mutations in *BRAF* (exon 15, NM_004333.6), *HRAS* (exons 2, 3, NM_005343.4), *KRAS* (exons 2, 3, NM_033360.4), *NRAS* (exons 2, 3, NM_002524.5) and *TP53* (exon 6, NM_000546.5) genes was performed in all samples as described previously¹⁶. DNA was amplified using gene-specific primers that are available with PCR conditions on request. The PCR products were purified using Agencourt AMPure (Beckman Coulter), libraries were prepared using a Nextera XT DNA Library Prep Kit (Illumina) according to the manufacturer's protocol and sequenced for 500 cycles by MiSeq Reagent Kit V2 (Illumina) using a MiSeq sequencer (Illumina). Integrative Genomics Viewer was used for sequence data visualization.

Detection of *TERT* mutations in FFPE samples

The detection of point mutations in *TERT* (promoter, NM_198253.3) gene was performed in all samples as described previously¹⁶. The PCR products of *TERT* promoter were purified using Agencourt AMPure (Beckman Coulter), and then sequencing reaction using Quick Start Master Mix Kit with Dye Terminator Cycle Sequencing (Beckman Coulter) was performed. Products of the sequencing reaction were purified using Agencourt Clean SEQ Dye-Terminator Removal (Beckman Coulter). Capillary sequencing was performed by CEQ 8000 instrument (Beckman Coulter). Analyses were evaluated by CEQ 8000 software (Beckman Coulter). The primer sequences and PCR conditions are available on request.

Table 1. Overview of patients, procedures and examination results.

Patient	Sex (M/F)	Age (years)	USG sn/mn [mm]	TI-RADS	FNAB Bethesda classification	Surgery TTE/HTE	Histo-patho- logical dg.	Molecular analysis (mutated gene)	Follow-up (months)
1	M	61	mn 17×15×22 19×16×19	4b	III. AUS/FLUS	TTE	NIFTP	<i>NRAS</i>	18
2	M	41	sn 67×48×35	2	II. Benign	HTE	NIFTP	<i>HRAS</i> <i>TP53</i>	6
3	F	69	sn 35×30×25	3	II. Benign	HTE	FT-UMP	0	6
4	M	66	sn 30×25×14	4b	III. AUS/FLUS	TTE	FT-UMP	0	26
5	M	50	sn 31×25×26	5	II. Benign	TTE	FT-UMP	0	58
6	F	53	sn 32×30×43	2	II. Benign	HTE	WDT-UMP	0	60
7	M	48	sn 40×30×25	4c	III. AUS/FLUS	TTE	HTT	<i>PAX8/GLIS3</i>	26
8	M	38	sn 40×27×27	2	I. Non-diagnostic	HTE	NIFTP	<i>NRAS</i>	14

M, male; F, female; mn, multinodular nodosity; USG, ultrasonography; sn, solitary nodosity; HTE/TTE, hemithyroidectomy/total thyroidectomy; FNAB, Fine Needle Aspiration Biopsy; AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; NIFTP, non-invasive follicular thyroid neoplasm with papillary-like nuclear features; HTT, hyalinizing trabecular tumour; FT-UMP, follicular tumour of uncertain malignant potential; WDT-UMP, well differentiated tumour of uncertain malignant potential; 0, no somatic pathogenic variant in the tested genes (*BRAF*, *HRAS*, *KRAS*, *NRAS*, *TERT*, *TP53*) or the presence of the tested *PAX8/PPARG* fusion gene was detected in the examined material; TI-RADS categories: TI-RADS 2, very low suspicion; TI-RADS 3, low suspicion; TI-RADS 4a, middle suspicion; TI-RADS 4b, 4c, 5, high suspicion.

Detection of *PAX8/GLIS3* and *PAX8/PPARG* fusion gene

In samples without detected driver mutation, analysis of *PAX8/PPARG* fusion genes (the fusion of exon 8, 9 or 10 of *PAX8* gene (NM_003466.3) with exon 2 of *PPARG* gene (NM005037.5) was performed. In HTT, the presence of the *PAX8/GLIS3* fusion was analysed (the fusion of exon 2 of the *PAX8* gene (NM_003466.3) with exon 3 of the *GLIS3* gene (NM_001042413.2). The analysis of the fusion genes was performed by Real-Time PCR. First, RNA was reverse transcribed into cDNA using random primers, AMV reverse transcriptase, RNase inhibitor, and dNTPs reagents. Subsequently, the cDNA was amplified using Sso Advanced Universal SYBR Green Supermix (Bio-Rad) and gene-specific primers. The primer sequences and PCR conditions are available on request. Real-Time PCR was performed using a Light Cycler 480 (Roche) and data were evaluated using a Light Cycler 480SW1.5.1. (Roche).

Statistical analysis

Categorical data are presented as counts and percentages, continuous data are described by means and SD. Between-group comparisons were carried out using Fisher's exact test and two-sample t-tests for categorical and continuous data respectively. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Demographic data, preoperative clinical and cytological findings, extent of surgery, postoperative histopathological and molecular genetic results are shown in Table 1.

A total of 487 patients were operated on during the study period. Benign thyroid diseases were found in 388

patients (79.7%), and thyroid carcinomas occurred in 91 (18.7%) patients.

BTT were found in a total of 8 operated patients (1.6%). This cohort of patients was characterized by a predominance of men (6 men, 2 women).

The mean age of patients with BTT was 54.36 years (range 38–69 years), in other patients it was 53.7 years (range 19–83 years), the difference was not statistically significant (*P*=0.919). Preoperative FNAB was performed in a total of 193 patients (39.6%) and classification into individual BSRTC categories was: a non-diagnostic/unsatisfactory sample (Bethesda I, 3.9%); a benign (Bethesda II, 20.3%); atypia/follicular lesions of undetermined significance (Bethesda III -AUS / FLUS, 6.6%); follicular neoplasm/suspicious for a follicular neoplasm (Bethesda IV -FN/SFN, 4.5%), suspicious for malignancy (Bethesda V - SM, 3.3%) and malignant (Bethesda VI, 1%).

In the BTT group, there was once (12.5%) a non-diagnostic/insufficient sample (BI-ND/UNS), a benign finding (BII) in four cases (50%), and atypia/follicular lesions of undetermined significance (BIII-AUS/FLUS) in three patients (37.5%). Other categories BIV-VI of the BSRTC did not appear in this group.

Total thyroidectomy (TTE) was performed in 3 patients with the cytological classification BIII-AUS/FLUS and in a patient with the cytological classification Bethesda II, who underwent external radiotherapy of the neck for Hodgkin's lymphoma with involvement of the cervical lymph nodes before the operation.

Hemithyroidectomy (HTE) was performed in three patients with the Bethesda II category and in patients with a non-diagnostic cytological specimen. All procedures were without postoperative complications. THE was performed in 50.0% of patients.

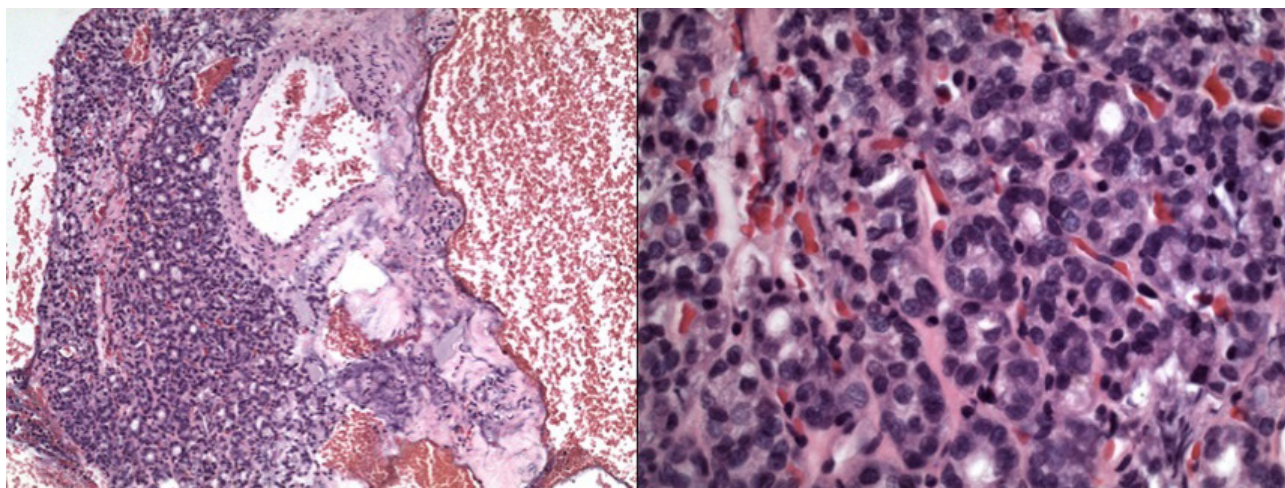


Fig. 1 A, B. FNAB cytoblock of the HTT case: Microphragment of a microfollicular homogeneous nodule with calcifications and FVPTC - like nuclei. Obj. magnif. 10× and 40×.

Molecular genetic testing

In three NIFTP patients pathogenic variants in *RAS* genes - c.182A>G p. (Gln61Arg) in exon 3 of *NRAS* gene in two cases and c.181C>A p. (Gln61Lys) in exon 3 of *HRAS* gene were detected. In NIFTP patient with *HRAS* mutation, a probable pathogenic variant c.586C>T (p.Arg196Ter) in exon 6 of *TP53* gene was detected. In a patient with HTT, a *PAX8/GLIS3* fusion gene was detected from the surgically removed tissue. In the remaining four cases (1 WDT-UMP and 3 FT-UMP), no somatic pathogenic variant in the analysed *BRAF*, *HRAS*, *KRAS*, *NRAS*, *TERT*, *TP53* genes associated with thyroid oncology, nor the presence of the *PAX8/PPARγ* fusion gene was detected (see Table 1).

Illustration case report

A male, 48-years old, euthyroid, observed a solitary node in the thyroid gland for about 4 months. Ultrasound examination revealed a right lobe 22×17×42 mm and a left lobe 19×16×47 mm. A voluminous hypoechogenic nodule 27×25×30 mm with numerous minor calcifications and increased vascularization was found in the isthmus part and right lower lobe reaching the upper edge of the sternum. According to ATA, high node suspicion, classification TI-RADS 4c (ref.¹⁷). Fine needle aspiration biopsy from a nodule under ultrasonography control was performed. Cytological evaluation revealed a microphragment of microfollicular tumour with calcifications and nuclei bearing some features of FVPTC - overlapping non-round nuclei with grooves and pseudoinclusions in Fig. 1. The cytopathology finding was classified as Bethesda III - AUS (atypia of undetermined significance) in the Bethesda system 2017 (ref.^{18,19}). CT examination confirmed an enlarged isthmus of 21–26 mm and an inhomogeneous nodule of 28×25×29 mm with calcifications in Fig. 2. Histopathological verification was recommended. Total thyroidectomy was performed without postoperative complications. Histopathology investigation found a well-defined nodule with solid alveolar and trabecular arrangement. The cells had voluminous eosinophilic cytoplasm

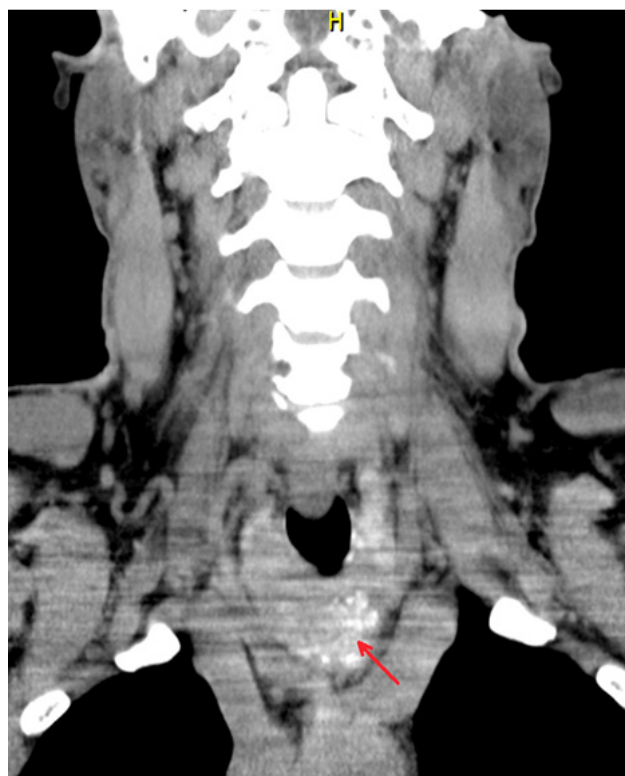


Fig. 2. CT scan: Enlarged isthmus of the thyroid gland 21–26 mm, which reaches the edge of the sternum with inhomogeneous nodule 25×25×29 mm and calcifications.

of oncocytic appearance. There were numerous calcifications and an abundant vascularization in the tumour. Cell nuclei were irregular with intranuclear pseudoinclusions and grooves. Immunohistochemistry revealed positive chromogranin, TTF1, CD 56, CD 31 positive in blood vessels, synaptophysin, Ki 67 in 1% and S100 negative. Angioinvasion was not shown and amyloid staining was negative. In the primary evaluation, medullary carcinoma was suspected and a consultation examination with an extension of the immunohistochemical panel was requested.

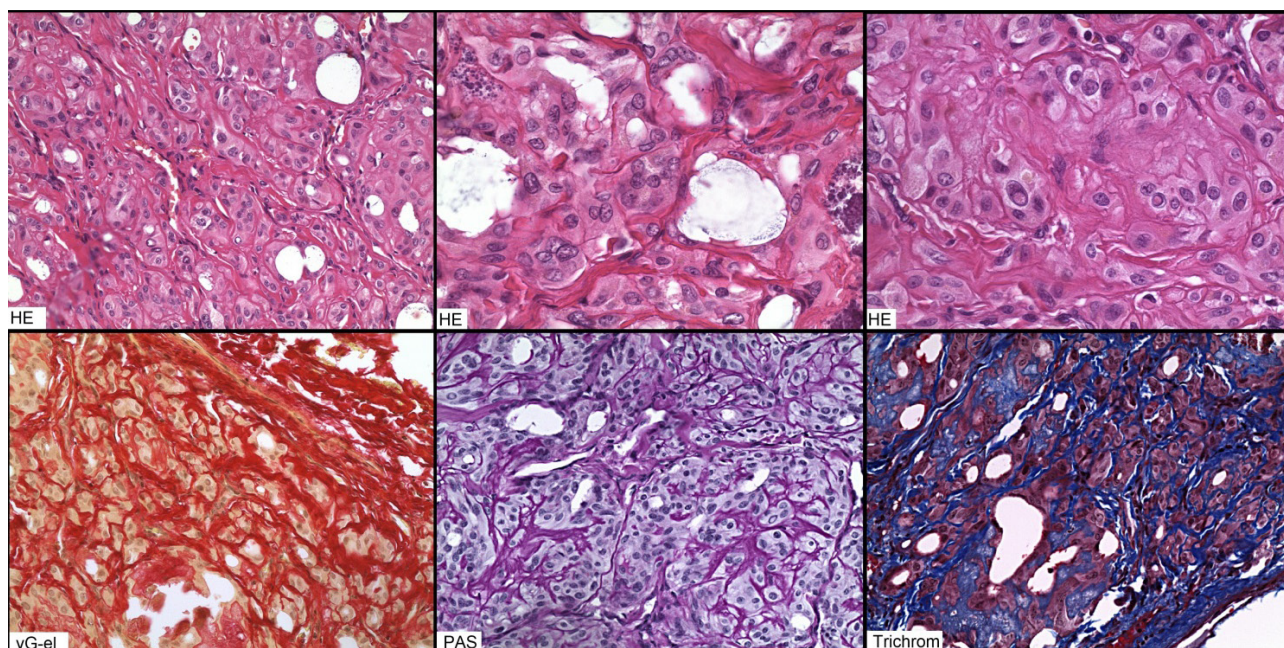


Fig. 3. Histopathology of the resectate. Solid trabecular and microfollicular arrangement with inter- and intratrabecular fibrosis. Nuclei with the features of PTC nuclei. HE, vG - el, trichrome, PAS staining. Obj. magnif. 20× and 40×.

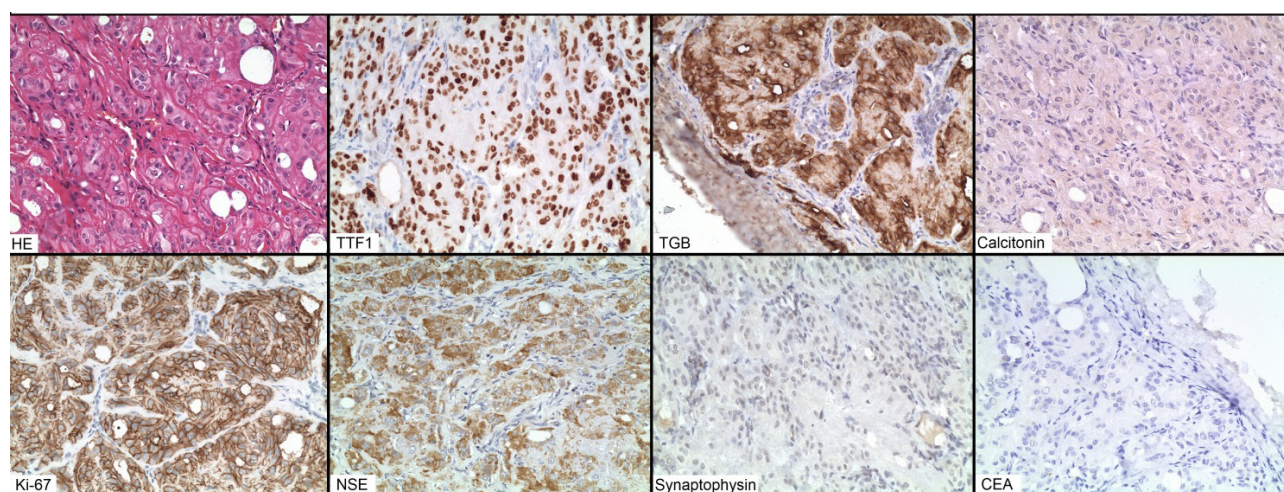


Fig. 4. Immunohistochemistry. TTF1, TGB, NSE - positive. Calcitonin, synaptophysin, CEA - negative. Ki-67 (MIB1) with typical for HTT membranous positivity. Obj. magnif. 20×.

Additional immunohistochemical examination was performed. Calcitonin and CEA were negative; thyroglobulin and TTF1 were diffusely positive, NSE had discrete positivity. Ki-67 (MIB1) exhibited membrane positivity of neoplastic cells. In relation to the morphological features (inter- and intratrabecular fibrosis, papillary carcinoma-like nuclei) and immunohistochemical profile (TTF1+, calcitonin-, Ki-67 (MIB1) - membrane positivity) second reading determined the final diagnosis of hyalinizing trabecular tumour in Fig. 3,4.

Molecular analysis of fusion genes from FFPE by Real-Time PCR (iQ SYBR Green Supermix, BIO-RAD, Light Cycler 480, Roche) detected a *PAX8/GLIS3* fusion gene (fusion of exon 2 of *PAX8* gene with exon 3 of *GLIS3* gene).

DISCUSSION

Individual variants of newly classified BTT differ in clinical behaviour, prognosis and molecular profile^{2,15} and may lead to a false positive diagnosis of papillary or medullary carcinoma^{15,19}.

In recent years, laboratories worldwide have made enormous effort to search for molecular markers that could preoperatively distinguish benign from malignant thyroid nodules²⁰. There are two main approaches, rule-out and rule-in analysis, or a combination of both. The aim of the rule-out approach is to identify benign nodules based on expression analysis of microRNA or DNA methylation profiling and to avoid unnecessary surgery. On the other hand, rule-in approach efforts to identify malignant nodules by detecting mutations specific for malignancy, leading to recommendation of the extent of surgery²¹.

Several molecular markers were identified. The most common genetic change of thyroid carcinomas is V600E mutation in the *BRAF* gene that is associated with 100% risk of malignancy. This mutation is specific for papillary thyroid carcinoma (PTC) and poorly differentiated thyroid carcinoma (PDTC) or anaplastic thyroid carcinoma (ATC) dedifferentiated from PTC (ref.²¹). Mutations in the promoter of the *TERT* gene occurring in all types of thyroid malignant tumours are associated with almost 100% risk of malignancy as well as presence of *RET*, *NTRK*, *ALK* or *BRAF* fusion genes²².

In postoperative histopathological diagnosis, immunohistochemically detectable markers - thyroid transcription factor-1 (TTF-1) contribute to confirm the origin of thyroid or to detect malignancy e.g. calcitonin in suspected medullary carcinoma, to a certain extent galectin 3, mesothelial 1 (Hector Battifora Mesothelial cell, cytokeratin-19).

Other markers are also proposed, e. g. CITED1, S100A, fibronectin-1, but so far without further detailed information²³. In our cohort, *RAS* mutations were detected in all three cases of NIFTP. *NRAS* and *HRAS* mutations are somatic pathogenic variants detected in exons 2 or 3. *RAS* mutations have been detected in all types of thyroid tumours, both benign and malignant, well differentiated or undifferentiated, thus their clinical significance is still unclear²⁴. The risk of malignancy in FNAB with *RAS* mutation is reported between 57–87% of cases¹⁵. In borderline follicular tumours, somatic *RAS* mutations have a high prevalence (in 30–67% of cases) (ref.¹⁵).

Patel et al. hypothesize that pathogenic variants in *RAS* genes are early transformation in benign tumours and may be predisposed to progression to cancer²⁴.

In a NIFTP patient with *HRAS* mutation additional variant in *TP53* gene was detected. In databases the variant is considered to be pathogenic and it was confirmed in functional studies²². Their clinical significance in NIFTP is unclear, however in general, pathogenic variants in *TP53* gene are associated with more aggressive tumour behaviour. In revised diagnostic criteria for NIFTP, absence of *TP53* mutations is recommended but not required for NIFTP diagnosis and the presence of such high-risk mutations should trigger an exhaustive search for invasive features and papillary formations and more careful follow-up of patients⁶.

In four cases, we did not detect any of the analysed somatic pathogenic variants of the tested genes. Similar results are reported by other authors^{21,25}.

In most cases, these are low-risk tumours with a favourable prognosis. The risk of recurrence or metastasis is very low but data on the long-term outcome of these tumours are limited. Completely removed lesions pose a risk of recurrence less than 1% and thus less radical surgery as lobectomy is recommended²¹. During the five-year follow-up period, our monitored patient developed no lymph node or distant metastases.

French et al.²⁶ consider capsule thickness as a predictive marker of invasion. The thickness of the tumour capsule is significantly thinner in NIFTP compared to

non-invasive encapsulated FVPTC, where it is thicker than 0.2 mm and which increases the suspicion of malignancy. The size of the tumour does not constitute a criterion for the diagnosis of NIFTP (ref.^{6,27}).

HTT is macroscopically a well-circumscribed or encapsulated nodule, white coloured, mostly asymptomatic. HTT tumour cells show positive expression of TTF-1, thyroglobulin, characteristic membrane positivity of Ki-67 (MIB1), with negative expression of calcitonin¹⁰. Ki-67 clone MIB1 helps to confirm HTT only with this clone and under defined conditions²⁸.

The aetiology of the tumour is not entirely elucidated. The cytological characteristics are similar to papillary carcinoma. It is considered by some authors to be a morphological variant of PTC, with overlapping nuclear changes, chromatin clarification and a high prevalence of *RET/PTC* rearrangement^{29,30}. Activation of the *RET/PTC* gene and production of the RET protein are involved in the nuclear changes that are typical of PTC. Some studies reported presence of *RET/PTC* rearrangement also in other tumours and lesions, such as lymphocytic thyroiditis³¹. However subsequent studies did not confirm the presence of *RET/PTC* rearrangement in HTT or other lesions except of PTC. According to the literature, only 8% of preoperative diagnoses of HTT are determined on the basis of cytological analysis^{30,32}. This possibility should be considered in the differential diagnosis more frequently^{32,33}. The absence of the *BRAF* mutation in HTT questions its association with PTC (ref.^{34,35}). The presence of hyaline material in HTT can be mistaken for amyloid and lead to a differential diagnostic consideration of medullary carcinoma^{10,30}. The situation was similar in our case of a patient with HTT (see the case report). In a recent study based on sequence analysis and other techniques, Nikiforova et al. detected a new *PAX8/GLIS3* fusion gene that is believed to be specific for HTT and could become key in the preoperative diagnosis of HTT^{10,29}. Nikiforova et al. consider the finding of the *PAX8/GLIS3* fusion gene as a specific marker for the diagnosis of HTT (ref.¹¹).

CONCLUSION

BTT are lesions with borderline histological findings between benign and malignant thyroid tumours and in most cases with indolent clinical behaviour. During the five-year follow-up period, no monitored patient developed lymph node or distant metastases. However, even with this favourable profile, surgical treatment is necessarily individual. It is influenced by preoperative clinical, ultrasonographic, cytological and molecular genetic findings, as well as the presence of other comorbidities. The definitive diagnosis is made on the basis of postoperative histopathological examination. Molecular genetic analysis contributes to the diagnosis of these lesions, and detection of the *PAX8/GLIS3* fusion gene is considered a specific finding in HTT.

ABBREVIATIONS

FNAB, Fine Needle Aspiration Biopsy; WHO, World Health Organization; TSH, Thyroid stimulating hormone; EFVPTC, Encapsulated Follicular Variant of Papillary Thyroid Carcinoma; TI-RADS, Thyroid Imaging Reporting and Data System; BSRTC, Bethesda System for Reporting Thyroid Cytopathology; ATA, American Thyroid Association.

Acknowledgements: This work was supported by Internal Grant from the Ministry of Health of the Czech Republic, Na Homolce Hospital – NNH, 00023884, IG192301 and was approved by the Ethical Committee of the NNH. Supported by Charles University – Progres Q28/LF1 project Supported by the Ministry of Health of the Czech Republic – RVO project VFN64165 and by MH CZ – DRO (Institute of Endocrinology – EÚ, 00023761).

Author contributions: JL: surgeon and conceptualization, manuscript writing; JD: conceptualization, final approval and supervision; VS: data analysis, following-up, final approval; BH: data analysis; MS: histological verification; MM: statistician.

Conflicts of interest statement: The authors have no conflict of interest to declare.

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