RESEARCH

Revised criteria for diagnosis of NIFTP reveals a better correlation with tumor biological behavior

Ana Carolina de Jesus Paniza[1], Thais Biude Mendes[1], Matheus Duarte Borges Viana[1], Débora Mota Dias Thomaz[1], Paula B O Chiappini[2], Gabriel A Colozza-Gama[1], Susan Chow Lindsey[3], Marcos Brasilino de Carvalho[4], Venâncio Avancini Ferreira Alves[5], Otavio Curioni[4], André Uchimura Bastos[1,6] and **Janete Maria Cerutti[1]**

[1]Division of Genetics, Department of Morphology and Genetics, Genetic Bases of Thyroid Tumors Laboratory, Universidade Federal de São Paulo, São Paulo, Brazil
[2]Department of Pathology, Hospital Heliópolis, São Paulo, Brazil
[3]Division of Endocrinology, Department of Medicine, Laboratory of Molecular and Translational Endocrinology, Universidade Federal de São Paulo, São Paulo, Brazil
[4]Department of Head and Neck Surgery and Otorhinolaryngology, Hospital Heliópolis, São Paulo, Brazil
[5]Department of Pathology, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil
[6]Department of Microbiology, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil

Correspondence should be addressed to J M Cerutti: j.cerutti@unifesp.br

Abstract

The recent reclassification of a follicular variant of papillary thyroid carcinoma (FVPTC), subset as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), aims to avoid overtreatment of patients with an indolent lesion. The diagnosis of NIFTP has recently been revisited using more rigid criteria. This study presents histological and molecular findings and a long clinical follow-up of 94 FVPTC, 40 cases of follicular adenoma (FTA) and 22 cases of follicular carcinoma (FTC) that were classified before the advent of the NIFTP reclassification. All slides were reviewed using these rigid criteria and analysis of numerous sections of paraffin blocks and reclassified as 7 NIFTPs, 2 EFVPTCs, 29 infiltrative FVPTC (IFVPTCs), 57 invasive EFVPTC (I-EFVPTCs), 39 FTAs and 22 FTCs. Remarkably, EFVPTC and NIFTP patients were all free of disease at the end of follow-up and showed no BRAF mutation. Only one NIFTP sample harbored mutations, an *NRAS* Q61R. *PAX8/PPARG* fusion was found in I-EFVPTCs and FTC. Although additional studies are needed to identify a specific molecular profile to aid in the diagnosis of lesions with borderline morphological characteristics, we confirmed that the *BRAF* V600E mutation is an important tool to exclude the diagnosis of NIFTP. We also show that rigorous histopathological criteria should be strongly followed to avoid missing lesions in which more aggressive behavior is present, mainly via the analysis of capsule or vascular invasion and the presence of papillary structures.

Key Words
- papillary thyroid carcinoma
- NIFTP
- BRAF V600E
- RAS
- PAX8-PPARG

Endocrine Connections (2019) **8**, 1529–1538

Introduction

The incidence of thyroid cancer has increased over the past decades and is predicted to continue to increase. Although a substantial proportion of the increase is attributable to an improved ability to detect small papillary thyroid carcinoma (PTC), the increase can also be partially ascribed to the evolution of histological criteria for the diagnosis of follicular variant of papillary thyroid carcinoma (FVPTC) (1, 2, 3, 4, 5, 6). This variant is composed predominantly of follicles with tumor cells having the nuclear features of PTC. Historically, the tumor capsule in this variant has been considered an important feature and has been regularly used to sort FVPTC into two

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-19-0459

main subtypes: encapsulated FVPTC (EFVPTC), when the tumor is surrounded by a fibrous capsule, and infiltrative FVPTC (IFVPTC), when there is no capsule.

For EFVPTC without invasion of the capsule or its vessels, the diagnosis of malignancy relies solely on the presence of the nuclear features of PTC. The subjectivity of the diagnosis has been a challenge for pathologists and a source of high interobserver variability (1, 2, 7, 8, 9). Based on the fact that some EFVPTCs have an indolent nature and that some with capsular or vascular invasion are prone to metastasis, some pathologists have divided the EFVPTC group into noninvasive EFVPTC (EFVPTC) and invasive EFVPTC (I-EFVPTC), according to the presence or absence of capsule and/or vascular invasion (10, 11).

As a subset of EFVPTC shows a very low lymph node metastasis risk and recurrence risk and has an excellent prognosis, reclassification of this tumor as a premalignant neoplasm was proposed in 2016 (12), and the new classification was termed noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTPs). This distinct entity, which is considered a 'premalignant' lesion, was included in the 2017 World Health Organization Classification of Tumors of Endocrine Organs book (13). However, distinct and strict anatomopathological characteristics needed to be observed to safely classify a tumor as a NIFTP, which introduced a new challenge.

The main criteria for the diagnosis of NIFTP (12) were revisited in 2018 with some adjustments (14, 15). Mainly, all NIFTPs must have a whole capsule involving the tumor or a well-demarcated parenchyma transition and must not have any type of invasion (neither capsular nor vascular), in addition to the follicular growth pattern and the presence of the nuclear features of PTC. Although papillary structure of up to 1% was initially accepted, in the revised NIFTP nomenclature, the absence of papillae is required (12, 16, 17, 18). Additionally, strict cytomorphonuclear features (typically more subtle than FVPTC and mainly localized to the periphery of the neoplasm), no psammoma bodies, no necrosis, and no more than three mitoses per 10 high-power fields (400×) are the most recent criteria used for this diagnostic category (14).

Based on the observed outcome data, the description of this new entity aimed to avoid overtreatment of an indolent tumor, suggesting lobectomy as satisfactory treatment, with no other additional adjuvant therapy (6, 12, 14, 16, 19, 20, 21, 22).

Considering the low malignancy potential of NIFTP and the fact that its diagnosis is postsurgical and requires complete histopathological analysis of surgical specimens to exclude invasion, it is challenging to distinguish NIFTP from EFVPTC and I-EFVPTC presurgically. Several groups have suggested that the same histopathological analysis used to distinguish follicular thyroid adenoma (FTA) and follicular thyroid carcinoma (FTC) could be used for NIFTP and I-EFVPTC. Additionally, it is suggested that the biological behavior of NIFTP is similar to that of FTA and infiltrative FVPTC, on the other hand, tends to clinically behave more like the classical variant of PTC, with a higher incidence of cervical lymph node metastasis (1, 17, 18, 23, 24, 25, 26, 27, 28, 29).

Recently, some groups have examined whether the molecular profiles observed in FTA, FTC and the classical variant of PTC could help in the diagnosis of NIFTP. As expected, mutations in *RAS* (mainly in codon 61 of *NRAS* and *HRAS*) are the most prevalent mutations found in NIFTP. *PAX8/PPARG* and THADA fusions and *EIF2AX* and BRAF K601 mutations were recently described in a small portion of NIFTPs (30, 31). The presence of the BRAF-V600E mutation may be a hint toward the presence of more aggressive histological features or even the presence of papillae and help to exclude the diagnosis of NIFTP (18, 26, 31, 32, 33, 34, 35, 36). As there is no association between a specific molecular profile and the diagnosis of NIFTP, mutation detection panels have not been used in the presurgical diagnosis of this entity.

Therefore, in this study, we applied the newest NIFTP classification criteria (14, 15) to a cohort of samples diagnosed as FVPTC prior to such recommendations. Then, we sought to identify the most common mutations found in FVPTC and correlated these findings, aiming to ascertain whether the stricter criteria would better correlate with the molecular profile, restrain overdiagnosis of malignancy and further assist in clinical management decisions.

Methods

Sample selection

This study was performed on available formalin-fixed, paraffin-embedded (FFPE) tissues selected from patients with thyroid tumors who underwent surgical resection from the year 2000 to 2015 at Hospital Heliópolis, São Paulo, Brazil and the material was available. Per the protocol of the department of pathological anatomy of this hospital, the entire tumor capsule was included for histopathological analysis at the time of macroscopic examination. To confirm the histological diagnosis and

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-19-0459

to ensure that representative tumor material was present, the routine hematoxylin and eosin (H&E)-stained sections were reviewed by a specialized pathologist (ACJP). The series comprised 156 FFPE tumor samples that were initially diagnosed as FVPTC (n=94), FTA (n=40) and FTC (n=22). The diagnostic criteria for these entities were based on previously described definitions (9).

For all specimens, serial tissue sections were cut from the selected paraffin block. DNA and RNA were isolated from three 10-µm-thick serial sections collected between step-sectioning levels 2 and 4. Ten 4-µm-thick sections collected between step-sectioning levels 5 and 14 were cut for FISH analysis or diagnostic stains. The first and last sections from each tissue block (superficial and about 100 µm deeper) were placed on one microscopic slide, stained with H&E and used for histological evaluations.

Clinical parameters such as age at diagnosis, sex and type of surgery were compiled. Follow-up was performed by the head and neck surgery team with serial measurements of serum thyroglobulin (Tg), thyroglobulin antibodies, and TSH; examination by neck ultrasound; and examination by whole-body scan (WBS) with ^{131}I when necessary.

This study was approved by the Hospital Heliopolis and Universidade Federal de São Paulo Review Board and Research Ethical Committee (1216/2015). Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Revision of tumor nomenclature

With the recent reclassification of an EFVPTC subset as NIFTP, the pathological parameters were subsequently revised according to the criteria defined and revised by Endocrine Pathology Society working group in 2016 (12) and 2018 (14, 15) (Post-NIFTP era) (Fig. 1). Tumor size, capsule status, parenchyma transition, multifocality, extrathyroidal extension and vascular invasion were analyzed. Cases with irregular parenchyma transition, any papillae, psammoma body, mitosis (more than 3 in 10 high-power fields) and necrosis were excluded from the diagnosis of NIFTP (Table 1). Nuclear features were carefully analyzed, as suggested by that working group, looking for enlargement, elongation, and overlap; pseudoinclusions, grooves, folds and irregular contours in membrane; and chromatin characteristics such as 'glassy' nuclei, delicate chromatin, chromatin margination to the membrane, and clearing.

DNA extraction and mutational profile of *BRAF*, *NRAS* and *HRAS*

For all specimens, the presence of *BRAF* (V600E and K601E), *NRAS* Q61, and *HRAS* Q61 mutations was evaluated in representative tumor sections. To avoid low tumor cell content, particular care was taken to select blocks comprising a tumor cell composition of at least 70%. Genomic DNA from each tumor was isolated from three 10 µm-thick FFPE sections using a NucleoSpin Tissue Kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions and quantified using a Nanodrop Spectrophotometer (Thermo Fisher Scientific). For PCR analyses, DNA (100 ng) was used as a template in a 50-µL PCR mixture containing 1× PCR buffer, 1.5 mM $MgCl_2$,

Figure 1
Sankey diagram to visualize histotype distribution changes across the different classification approaches. (A) Original diagnostic: FVPTC (n = 94), FTA (n = 40) and FTC (n = 23). (B) Histotypes assignment was performed according to criteria defined by Seethala *et al.* (14). The samples classified as NIFTPs were previously classified as EFVPTC (6 out of 7) or FTA (1 out of 7). Most EFVPTC were reclassified as I-EFVPTC (n = 15) or IFVPTC (n = 1). None of the FTC cases changed category.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-19-0459

Table 1 Anatomopathological and molecular features of NIFTP and EFVPTC samples.

ID	Age (year)	Sex	Size (cm)	Papillae	Capsule invasion	Vascular invasion	Multifocality	ETE	Necrosis	Mitosis	LNM	Mutational profiling	Reclassification[a]
1	40	F	1.5	No	No	No	No	No	No	No	No	Negative	NIFTP
2	51	F	3.0	No	No	No	No	No	No	No	No	Negative	NIFTP
3	50	F	3.5	No	No	No	No	No	No	No	No	Negative	NIFTP
4	82	F	1.0	No	No	No	No	No	No	No	No	Negative	NIFTP
5	49	F	1.0	No	No	No	No	No	No	No	No	Negative	NIFTP
6	34	F	2.0	No	No	No	No	No	No	No	No	NRAS Q61R	NIFTP
7	34	M	3.0	Yes	No	No	No	No	No	No	No	Negative	EFVPTC
8	37	F	2.5	Yes	No	No	No	No	No	No	No	Negative	EFVPTC

[a]According to the revision published in 2018 (14).
EFVPTC, encapsulated follicular variant of papillary thyroid carcinoma; ETE, extrathyroidal extension; LNM, lymph node metastasis; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features.

200 µM dNTPs, 100 nM of each primer (Supplementary Table 1, see section on supplementary data given at the end of this article) and 2 U of Platinum Taq DNA Polymerase (Thermo Fisher Scientific).

For amplification of exon 15 of *BRAF*, the PCR protocol was performed as previously described: an initial denaturation step at 94°C for 5 min followed by 40 cycles of a three-step program, namely, 94°C for 30 s, 53°C for 40 s and 72°C for 30 s, and a final 2-min extension at 72°C (37). For amplification of exon 2 of *NRAS* or *HRAS*, the amplification protocol was an initial denaturation step at 94°C for 5 min followed by 40 cycles of a three-step program, namely, 94°C for 30 s, 56°C for 45 s and 72°C for 45 s, and a final 7-min extension at 72°C.

The PCR products were resolved on a 2% agarose gel, visualized on a Bio-Rad Gel Doc EZ system (Bio-Rad) and purified using illustra ExoProStar S (GE Healthcare). Purified products were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific). Each sample was sequenced at least twice.

RNA extraction, cDNA synthesis and *PAX8/PPARG* screening

RNA was isolated from three 10-µm-thick FFPE sections using the Recover All Total Nucleic Acid Isolation kit (Thermo Fisher Scientific) following the manufacturer's instructions. Total RNA (500 ng) was reverse transcribed into cDNA, and quality was determined by amplification of the internal control gene (*RPS8*) as previously described (38). Screening for *PAX8/PPARG* rearrangement (fusion of exon 9 of *PAX8* and exon 2 of *PPARG*) was performed by quantitative RT-PCR. Briefly, cDNA was synthesized as previously described (39) and subjected to PCR amplification using the TaqMan assay (Assay ID Hs04396714_ft, Thermo Fisher Scientific). The quantitative PCR reaction was performed in triplicate, and the threshold cycle was obtained using QuantStudio™ 12K Flex Software v1.2.2 (Thermo Fisher Scientific). PCR products were analyzed by electrophoresis on a 2% agarose gel and visualized on a Bio-Rad Gel Doc EZ system (Bio-Rad).

Results

Nomenclature revision better correlates with the biological behavior of the tumor

The initial diagnosis of these cases was performed before the newly introduced pathological diagnosis of NIFTP (Fig. 1).

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-19-0459

Because NIFTP has emerged as a new category of thyroid tumors, with low, if any, potential of malignancy, and could therefore impact clinical management, we reassessed all samples according to the revision published in 2018 (14). In addition to routine slides, H&E-stained slides prepared from the first and last slide of the tissue in the block that was genotyped were extensively reviewed by two pathologists (A C J P and V A F A).

The revision of the available routine H&E staining and the new slides obtained from uncut material (superficial and about 100 µm deeper in the paraffin block) reclassified the tumors as follows: 6 NIFTPs, 2 EFVPTCs, 57 I-EFVPTCs and 29 IFVPTCs (Fig. 1).

A representative case of NIFTP is shown in Fig. 2A and B. Of note, the two samples that were diagnosed as EFVPTC were not classified as NIFTP because they presented a well-formed papillary structure in the center of the tumor (samples #7 and #8, Fig. 2C, D and Table 1, respectively), which, if not for these criteria, would raise the overall prevalence of NIFTP (Fig. 2D).

All cases of FTC were reanalyzed in light of the new classification proposed and maintained the diagnosis. Additionally, one FTA case was reclassified as NIFTP. This individual sample showed sufficient nuclear characteristics for a NIFTP diagnosis at the periphery of the lesion (Fig. 2E). Overall, the prevalence of NIFTP was 5% (Fig. 1 and Table 2).

These data highlight the importance of deeper sections for a reliable diagnosis, mainly when invasion through the tumor capsule or vascular invasion are investigated for a diagnosis of malignancy (14).

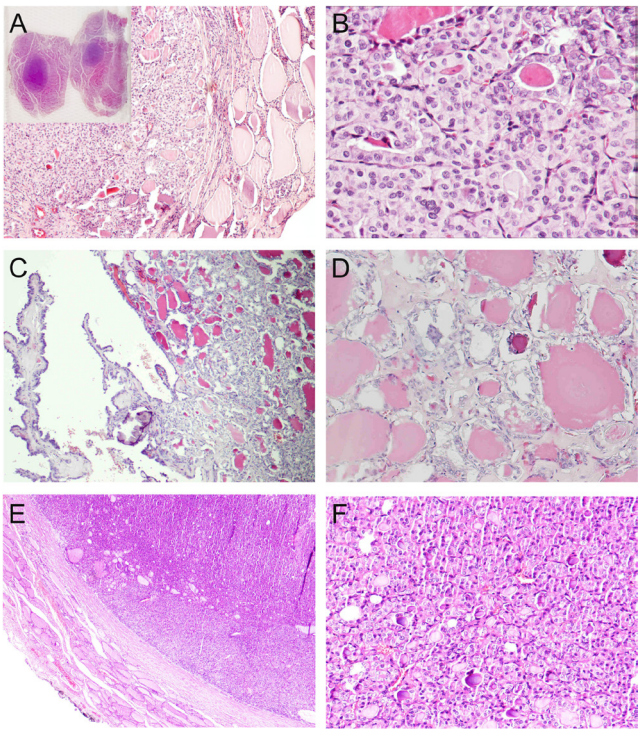

Figure 2
Histology of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTPs) and encapsulated follicular variant of papillary thyroid carcinomas (EFVPTC). (A and B) Representative image of a NIFTP sample showing the characteristics necessary for the diagnosis: (A) an expansive growth pattern with a well-delineated interface with the surrounding thyroid parenchyma and the absence of an invasion signal. This well-delimited interface can be noted even macroscopically (detail). (B) In addition, NIFTP samples must have papillary-like nuclei, as in this photomicrography. (C and D) EFVPTC cases excluded from the NIFTP group due to the presence of true papillae in the middle of the follicular architecture: (C) sample #9 and (D) sample #10, according to Table 1. (E and F) FTA sample whose diagnosis was changed to NIFTP: (E) a thick capsule can be seen around the tumor. There are no signs of invasion of the vessel or capsule, and only follicular architecture is observed. (F) At the periphery of the lesion, however, the nuclei show irregular membranes, overlap, grooves, indentations and chromatin clearing, which are sufficient criteria for the diagnosis of NIFTP (Hematoxylin-eosin, original magnifications of 40x (A and E), 400× (B), 200× (C, D and F).

The BRAF V600E mutation in the post-NIFTP era

We observed that the mutational rate is higher in IFVPTC (59%) than in I-EFVPTC (46%) (Fig. 3A). The BRAF V600E mutation was found only in samples that showed invasion, that is, in I-EFVPTC ($n=16$; 28%) and IFVPTC ($n=13$; 45%). Remarkably, one NIFTP sample harbored an *NRAS* Q61R mutation, and none of the other genetic events investigated in this work were found in the other six NIFTP samples.

Mutational profile of FTAs and FTCs

Since NIFTPs are frequently associated with a similar mutational profile to those of FTA and FTC, we investigated the prevalence of the same alterations in 39 FTAs and 22 FTCs, as one FTA had been reclassified as NIFTP. As shown in Fig. 3B, most FTAs and FTCs harbored mutations in the *RAS* gene.

All NIFTPs have an indolent clinical disease course

The central goal of the reclassification of some FVPTCs as NIFTPs is to avoid overtreatment of patients with an indolent tumor. When we evaluated the clinical and pathological features at the time of surgery, no case of NIFTP showed metastasis at diagnosis. In the present study the frequency of NIFTP was 5% (7/134).

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-19-0459

Table 2 Follow-up information of the NIFTP and EFVPTC samples.

ID[a]	Reclassification	Mutation	Treatment	Surgery (date)	Structural disease	Biochemical disease	End-of-follow-up findings	Time of final visit (years)
1	NIFTP	Negative	TT	2006	No	No	No evidence of disease	11.4
2	NIFTP	Negative	TT + RAI	2005	No	No	No evidence of disease	12.5
3	NIFTP	Negative	TT + RAI	2006	No	No	No evidence of disease	4.8
4	NIFTP	Negative	LB	2009	NA	NA	Dead by other causes	2.0
5	NIFTP	Negative	LB	2015	No	No	No evidence of disease	3.0
6	NIFTP	NRAS Q61R	TT + RAI	2006	No	No	No evidence of disease	9.7
7	EFVPTC	Negative	LB	2001	No	No	No evidence of disease	8.9
8	EFVPTC	Negative	TT	2010	No	No	No evidence of disease	7.7
FTA[b]	NIFTP	Negative	LB	2003	NA	NA	Lost to follow-up	NA

[a]ID according to Table 1. [b]Sample originally classified as FTA that showed nuclear characteristics at the periphery of the lesion that met the NIFTP criteria. EFVPTC, encapsulated follicular variant of papillary thyroid carcinoma; NA, not available; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; LB, lobectomy; LCD, lateral compartment dissection; RAI, radioactive iodine ablation; TT, total thyroidectomy.

To evaluate the course of disease, we collected follow-up information for all FVPTCs, ranging from 0 to 14.8 years, with an average of 7.2 years of follow-up. Patients with NIFTP were followed for 2–12.5 years (mean, 7.5 years), and all of them were free of disease at the end of the follow-up period (Table 2), except for one patient who died from other causes.

Discussion

Several histological variants have been recognized, among which FVPTC is the second most common. Although the diagnosis of FVPTC has evolved over the last decade, it still presents a great dilemma for the pathologist in routine practice. The source of difficulty is the subjectivity involved in a substantial number of cases. Even when a panel of molecular markers is applied, the clear-cut categorization of FVPTC is still challenging (40). Extra complexity is attributed to divergent outcomes in EFVPTC, which contributes to the overdiagnosis and overtreatment of patients.

With the aim of a better subclassification of noninvasive EFVPTC, a multidisciplinary group of scientists reexamined this entity through a review of cases with extended follow-up periods. Based on clinical and pathological reports and the noninvasive nature of most cases, a new terminology named noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTPs) was proposed (12). The results of that study showed that when the diagnosis of NIFTP is made on meticulous inspection of key histological features, the tumor will have a very low recurrence rate, if any, over the next 15 years, and therefore, patients may benefit from lobectomy (12). It was projected that patients with tumors diagnosed with NIFTP are unlikely to benefit from complementation thyroidectomy, RAI therapy or

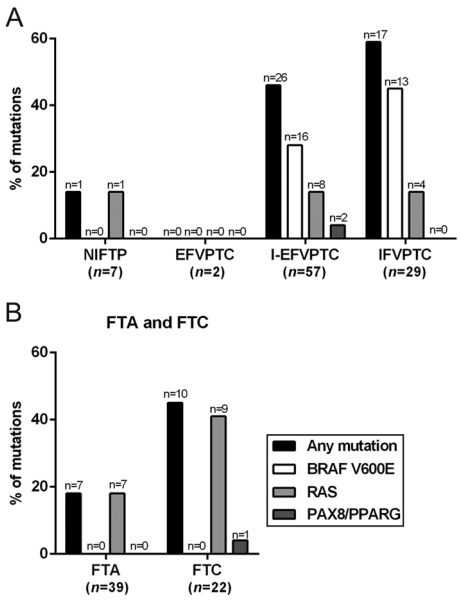

Figure 3
Percentage of the mutations in FVPTC across different classifications: (A) Post-NIFTP era. (B) Percentage of mutations in the FTA and FTC samples after histological reclassification.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-19-0459

intense follow-up, thus leading to lower complication rates associated with total thyroidectomy and to risk of secondary tumors following RAI (12, 26).

The present study originally evaluated 156 follicular lesions whose diagnoses were performed before the implementation of NIFTP, including 94 cases of FVPTC, 40 cases of FTA and 22 cases of FTC (Fig. 1).

To confirm the histological diagnosis and to perform molecular analysis, serial sections of the tissue were cut, and new slides, including sections that were superficial and about 100 µm deeper in the paraffin blocks, were reviewed in accordance with the recently published NIFTP criteria (14, 26).

Importantly, all samples that were metastatic at diagnosis showed some type of invasion (vascular or capsular) and were reclassified as I-EFVPTC (15 cases) or even as IFVPTC (one case). Six cases were classified as NIFTP; however, two cases showed a papillary structure, and therefore, reclassified as EFVPTC (Table 1). The absence of a papillary structure was recently applied as a rigid diagnostic criterion for NIFTP (14, 26, 41). This criterion was previously suggested in the study by Cho *et al.*, which showed that an arbitrary cutoff of less than 1% papillae could give rise to diagnostic discrepancies and lead to a misclassification of biologically more aggressive variants of PTC as NIFTP (42).

This extensive histopathological analysis, based on more rigid criteria, highlights the importance of careful pathological examination, as it is crucial to reveal vascular or capsule invasion and the presence of papillae.

Remarkably, in our samples, half of the BRAF V600E-positive I-EFVPTC samples showed some papillary structure, and although these samples were indeed invasive, these data add to the association between papillae and more aggressive characteristic (7, 42, 43).

Since NIFTP samples show subtle or focal nuclear features of PTC with a follicular morphology, the differential diagnosis between NIFTP and FTA could also be a challenge to pathologists (16, 44). Indeed, when meticulous histological analysis was performed, one case previously diagnosed as FTA showed nuclear characteristics at the periphery of the lesion that met the criteria required for NIFTP (score 2 of 3, defined in the nuclear score scheme by Nikiforov's group in 2018 (14)) and was reclassified. In this scoring system, one point is given every time a feature category is considered adequate in extent and quality. The categories are three: size and shape, membrane irregularities and chromatin characteristics. Two of the three categories should be adequate enough (i.e., score 2 of 3) to be able to diagnose NIFTP (14). In summary, the frequency of NIFTP in this study was 5% (7/134).

Although a few studies have demonstrated that a small percentage of patients with lesions fulfilling the diagnostic criteria for NIFTP develop regional metastases (42), in our study, none of the tumors classified as NIFTP showed recurrence during a mean follow-up period of 7 years (2–12.5 years).

Molecular genotyping of the samples is a strong tool that aids in the final diagnosis. Herein, we observed an interesting association between the presence of a tumor capsule and signs of tumor invasion with mutational profiling.

Approximately 50% of the FVPTC samples with any invasion harbored some type of mutation. Importantly, all samples that harbored the BRAF V600E mutation were I-EFVPTC or IFVPTC when reviewed by two experienced pathologists who were blinded to the mutation status, which corroborates the more aggressive phenotype associated with the BRAF V600E mutation. Two cases had BRAF V600E co-occurring with NRAS Q61R, a correlation widely described in the literature (17, 23, 26, 30, 45, 46, 47, 48, 49). No EFVPTC or NIFTP had *BRAF* mutations.

Importantly, most NIFTPs and, therefore, tumors with no papillary structure, were negative for the most common mutations previously found in PTC. These data suggest that a different driver mutation might be related to tumor initiation in a significant percentage of the NIFTP cases. Moreover, this study confirms that the BRAF V600E mutation could be an important tool for excluding the diagnosis of NIFTP. Thereafter, an immunohistochemistry study with the BRAFVE antibody, as part of a panel, may supplement architectural and morphological information (32).

As described for FTA and FTC, *NRAS* codon 61 mutations and *PAX8/PPARG* are the most common abnormality observed in FVPTC. Although the *NRAS* mutation is a very prevalent event in follicular thyroid lesions, this mutation does not show a statistical correlation with any specific lesion, as previously observed (21, 27, 30, 42, 45).

As NIFTP samples have been described to have a similar mutational profile to those of FTA, with frequent *RAS* gene mutations and *PAX8/PPARG* fusions, we here investigate the prevalence of RAS and *PAX8/PPARG* fusion. NRAS Q61K mutation was found in both NIFTP and I-EFVPTC. *PAX8/PPARG* rearrangement was found in two I-EFVPTCs with lymphatic vascular invasion and an FTC with capsular invasion. As previously described

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-19-0459

in the literature, this rearrangement is associated with signs of vascular or capsular invasion, although it is not a specific molecular finding of malignant nodules (12, 17, 49, 50).

In conclusion, the present study shows that careful postoperative histopathological evaluation continues to be fundamental for the diagnosis of lesions with follicular morphology, being reinforced after the advent of the NIFTP entity. The rigorous histopathological criteria should be strictly followed to avoid the neglect of lesions with more aggressive behavior. Another important point was that with our long clinical follow-up time, we confirmed that NIFTP has a more indolent disease course, adding to the already proposed less aggressive treatment performed for benign lesions. To date, this evaluation cannot be replaced by preoperative molecular tests as the mutational profiling of this indolent category is still not completely understood, but the molecular profile has proved to be a robust tool in confirming the diagnosis of more aggressive variants. Prospective additional studies in a larger population of cases with longer follow-up times are needed to define a specific molecular profile to aid in the diagnosis of lesions with borderline morphological characteristics.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/EC-19-0459.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

The study was supported by research grants from the São Paulo State Research Foundation (FAPESP) grant number 2014/06570-6. T B M (2017/06487-0), D M D T (2017/16315-1) and A U B (2016/25784-2) are recipients of fellowship grants from FAPESP. J M C is a recipient of a scholarship of Research Productivity from CNPq.

References

1 Gupta S, Ajise O, Dultz L, Wang B, Nonaka D, Ogilvie J, Heller KS & Patel KN. Follicular variant of papillary thyroid cancer: encapsulated, nonencapsulated, and diffuse: distinct biologic and clinical entities. *Archives of Otolaryngology: Head and Neck Surgery* 2012 **138** 227–233. (https://doi.org/10.1001/archoto.2011.1466)

2 Vivero M, Kraft S & Barletta JA. Risk stratification of follicular variant of papillary thyroid carcinoma. *Thyroid* 2013 **23** 273–279. (https://doi.org/10.1089/thy.2012.0369)

3 Nettore IC, Colao A & Macchia PE. Nutritional and environmental factors in thyroid carcinogenesis. *International Journal of Environmental Research and Public Health* 2018 **15** 1735. (https://doi.org/10.3390/ijerph15081735)

4 Jung CK, Little MP, Lubin JH, Brenner AV, Wells SA, Sigurdson AJ & Nikiforov YE. The increase in thyroid cancer incidence during the last four decades is accompanied by a high frequency of BRAF mutations and a sharp increase in RAS mutations. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E276–E285. (https://doi.org/10.1210/jc.2013-2503)

5 Xu B, Tallini G, Scognamiglio T, Roman BR, Tuttle RM & Ghossein RA. Outcome of large noninvasive follicular thyroid neoplasm with papillary-like nuclear features. *Thyroid* 2017 **27** 512–517. (https://doi.org/10.1089/thy.2016.0649)

6 Jeon MJ, Song DE, Jung CK, Kim WG, Kwon H, Lee YM, Sung TY, Yoon JH, Chung KW, Hong SJ, *et al.* Impact of reclassification on thyroid nodules with architectural atypia: from non-invasive encapsulated follicular variant papillary thyroid carcinomas to non-invasive follicular thyroid neoplasm with papillary-like nuclear features. *PLoS ONE* 2016 **11** e0167756. (https://doi.org/10.1371/journal.pone.0167756)

7 Rossi ED & Faquin WC. NIFTP revised: chronicle of a change foretold. *Cancer Cytopathology* 2018 **126** 897–901. (https://doi.org/10.1002/cncy.22070)

8 Xu B & Ghossein R. Encapsulated thyroid carcinoma of follicular cell origin. *Endocrine Pathology* 2015 **26** 191–199. (https://doi.org/10.1007/s12022-015-9376-5)

9 Asa A, De Jesus A, Kerr D, Mete O, Nosé V, Papotti M & Volante M. Thyroid. In *Endocrine Pathology*, pp 398–572. Cambridge, UK: Cambridge University Press, 2016.

10 Ganly I, Wang L, Tuttle RM, Katabi N, Ceballos GA, Harach HR & Ghossein R. Invasion rather than nuclear features correlates with outcome in encapsulated follicular tumors: further evidence for the reclassification of the encapsulated papillary thyroid carcinoma follicular variant. *Human Pathology* 2015 **46** 657–664. (https://doi.org/10.1016/j.humpath.2015.01.010)

11 Mete O & Asa SL. Pathological definition and clinical significance of vascular invasion in thyroid carcinomas of follicular epithelial derivation. *Modern Pathology* 2011 **24** 1545–1552. (https://doi.org/10.1038/modpathol.2011.119)

12 Nikiforov YE, Seethala RR, Tallini G, Baloch ZW, Basolo F, Thompson LD, Barletta JA, Wenig BM, Al Ghuzlan A, Kakudo K, *et al.* Nomenclature revision for encapsulated follicular variant of papillary thyroid carcinoma: a paradigm shift to reduce overtreatment of indolent tumors. *JAMA Oncology* 2016 **2** 1023–1029. (https://doi.org/10.1001/jamaoncol.2016.0386)

13 Lloyd RV, Osamura TY, Kloppel G & Rosi JE. *Classification of Tumours of Endocrine Organs*, 4th ed., vol 10. Lyon, France: IARC Press, 2017.

14 Seethala RR, Baloch ZW, Barletta JA, Khanafshar E, Mete O, Sadow PM, LiVolsi VA, Nikiforov YE, Tallini G & Thompson LD. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: a review for pathologists. *Modern Pathology* 2018 **31** 39–55. (https://doi.org/10.1038/modpathol.2017.130)

15 Nikiforov YE, Baloch ZW, Hodak SP, Giordano TJ, Lloyd RV, Seethala RR & Wenig BM. Change in diagnostic criteria for noninvasive follicular thyroid neoplasm with papillary like nuclear features. *JAMA Oncology* 2018 **4** 1125–1126. (https://doi.org/10.1001/jamaoncol.2018.1446)

16 Thompson LD. Ninety-four cases of encapsulated follicular variant of papillary thyroid carcinoma: a name change to noninvasive follicular thyroid neoplasm with papillary-like nuclear features would help prevent overtreatment. *Modern Pathology* 2016 **29** 698–707. (https://doi.org/10.1038/modpathol.2016.65)

17 D'Cruz AK, Vaish R, Vaidya A, Nixon IJ, Williams MD, Vander Poorten V, López F, Angelos P, Shaha AR, Khafif A, *et al.* Molecular markers in well-differentiated thyroid cancer. *European Archives of Oto-Rhino-Laryngology* 2018 **275** 1375–1384. (https://doi.org/10.1007/s00405-018-4944-1)

18 Paulson L, Shindo M, Schuff K & Corless C. The role of molecular markers and tumor histological type in central lymph node metastasis of papillary thyroid carcinoma. *Archives of Otolaryngology: Head and Neck Surgery* 2012 **138** 44–49. (https://doi.org/10.1001/archoto.2011.226)

19 Xu B, Tallini G & Ghossein RA. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: historical context, diagnosis, and future challenges. *Endocrine Pathology* 2017 **28** 128–138. (https://doi.org/10.1007/s12022-017-9478-3)

20 Rosario PW. Long-term outcomes of patients with noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) ≥4 cm treated without radioactive iodine. *Endocrine Pathology* 2017 **28** 367–368. (https://doi.org/10.1007/s12022-017-9493-4)

21 Paulson VA, Shivdasani P, Angell TE, Cibas ES, Krane JF, Lindeman NI, Alexander EK & Barletta JA. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features accounts for more than half of 'carcinomas' harboring RAS mutations. *Thyroid* 2017 **27** 506–511. (https://doi.org/10.1089/thy.2016.0583)

22 Haugen BR, Sawka AM, Alexander EK, Bible KC, Caturegli P, Doherty GM, Mandel SJ, Morris JC, Nassar A, Pacini F, *et al.* American Thyroid Association guidelines on the management of thyroid nodules and differentiated thyroid cancer task force review and recommendation on the proposed renaming of encapsulated follicular variant papillary thyroid carcinoma without invasion to noninvasive follicular thyroid neoplasm with papillary-like nuclear features. *Thyroid* 2017 **27** 481–483. (https://doi.org/10.1089/thy.2016.0628)

23 Howitt BE, Jia Y, Sholl LM & Barletta JA. Molecular alterations in partially-encapsulated or well-circumscribed follicular variant of papillary thyroid carcinoma. *Thyroid* 2013 **23** 1256–1262. (https://doi.org/10.1089/thy.2013.0018)

24 Zhu Z, Gandhi M, Nikiforova MN, Fischer AH & Nikiforov YE. Molecular profile and clinical-pathologic features of the follicular variant of papillary thyroid carcinoma. An unusually high prevalence of ras mutations. *American Journal of Clinical Pathology* 2003 **120** 71–77 (https://doi.org/10.1309/ND8D-9LAJ-TRCT-G6QD)

25 Rivera M, Ricarte-Filho J, Knauf J, Shaha A, Tuttle M, Fagin JA & Ghossein RA. Molecular genotyping of papillary thyroid carcinoma follicular variant according to its histological subtypes (encapsulated vs infiltrative) reveals distinct BRAF and RAS mutation patterns. *Modern Pathology* 2010 **23** 1191–1200. (https://doi.org/10.1038/modpathol.2010.112)

26 Nikiforov YE. Role of molecular markers in thyroid nodule management: then and now. *Endocrine Practice* 2017 **23** 979–988. (https://doi.org/10.4158/EP171805.RA)

27 Radkay LA, Chiosea SI, Seethala RR, Hodak SP, LeBeau SO, Yip L, McCoy KL, Carty SE, Schoedel KE, Nikiforova MN, *et al.* Thyroid nodules with KRAS mutations are different from nodules with NRAS and HRAS mutations with regard to cytopathologic and histopathologic outcome characteristics. *Cancer Cytopathology* 2014 **122** 873–882. (https://doi.org/10.1002/cncy.21474)

28 Mehrzad R, Nishino M, Nucera C, Dias-Santagata D, Hennessey JV & Hasselgren PO. Invasive follicular variant of papillary thyroid cancer harboring the NRAS mutation Q61K and presenting with bone metastasis – a case report. *International Journal of Surgery Case Reports* 2017 **38** 180–184. (https://doi.org/10.1016/j.ijscr.2017.06.067)

29 Hwang TS, Kim WY, Han HS, Lim SD, Kim WS, Yoo YB, Park KS, Oh SY, Kim SK & Yang JH. Preoperative RAS mutational analysis is of great value in predicting follicular variant of papillary thyroid carcinoma. *BioMed Research International* 2015 **2015** 697068. (https://doi.org/10.1155/2015/697068)

30 Pool C, Walter V, Bann D, Goldenberg D, Broach J, Hennessy M, Cottrill E, Washburn E, Williams N, Crist H, *et al.* Molecular characterization of tumors meeting diagnostic criteria for the non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). *Virchows Archiv* 2019 **474** 341–351. (https://doi.org/10.1007/s00428-018-02512-6)

31 Brandler TC, Liu CZ, Cho M, Zhou F, Cangiarella J, Yee-Chang M, Shi Y, Simsir A & Sun W. Does noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) have a unique molecular profile? *American Journal of Clinical Pathology* 2018 **150** 451–460. (https://doi.org/10.1093/ajcp/aqy075)

32 Johnson DN & Sadow PM. Exploration of BRAFV600E as a diagnostic adjuvant in the non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). *Human Pathology* 2018 **82** 32–38. (https://doi.org/10.1016/j.humpath.2018.06.033)

33 Rossi ED & Martini M. New insight in a new entity: NIFTPS and valuable role of ancillary techniques. The role of PD-L1. *EBiomedicine* 2017 **18** 11–12. (https://doi.org/10.1016/j.ebiom.2017.03.035)

34 Sahli ZT, Umbricht CB, Schneider EB & Zeiger MA. Thyroid nodule diagnostic markers in the face of the new NIFTP category: time for a reset? *Thyroid* 2017 **27** 1393–1399. (https://doi.org/10.1089/thy.2017.0238)

35 Zhao L, Dias-Santagata D, Sadow PM & Faquin WC. Cytological, molecular, and clinical features of noninvasive follicular thyroid neoplasm with papillary-like nuclear features versus invasive forms of follicular variant of papillary thyroid carcinoma. *Cancer Cytopathology* 2017 **125** 323–331. (https://doi.org/10.1002/cncy.21839)

36 Pennelli G, Vianello F, Barollo S, Pezzani R, Merante Boschin I, Pelizzo MR, Mantero F, Rugge M & Mian C. BRAF(K601E) mutation in a patient with a follicular thyroid carcinoma. *Thyroid* 2011 **21** 1393–1396. (https://doi.org/10.1089/thy.2011.0120)

37 Pinheiro dos Santos MJC, Bastos AU, da Costa VR, Delcelo R, Lindsey SC, Colozza-Gama GA, Peng H, Rauscher FJ, Oler G & Cerutti JM. LIMD2 is overexpressed in BRAF V600E – positive papillary thyroid carcinomas and matched lymph node metastases. *Endocrine Pathology* 2018 **29** 222–230. (https://doi.org/10.1007/s12022-018-9526-7)

38 Cordioli MI, Moraes L, Bastos AU, Besson P, Alves MT, Delcelo R, Monte O, Longui C, Cury AN & Cerutti JM. Fusion oncogenes are the main genetic events found in sporadic papillary thyroid carcinomas from children. *Thyroid* 2017 **27** 182–188. (https://doi.org/10.1089/thy.2016.0387)

39 Nakabashi CC, Guimarães GS, Michalauart PJr, Ward LS, Cerutti JM & Maciel RM. The expression of PAX8-PPARgamma rearrangements is not specific to follicular thyroid carcinoma. *Clinical Endocrinology* 2004 **61** 280–282. (https://doi.org/10.1111/j.1365-2265.2004.02061.x)

40 Amendoeira I, Maia T & Sobrinho-Simões M. Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP): impact on the reclassification of thyroid nodules. *Endocrine-Related Cancer* 2018 **25** R247–R258. (https://doi.org/10.1530/ERC-17-0513)

41 Rosario PW & Mourão GF. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP): a review for clinicians. *Endocrine-Related Cancer* 2019 **26** R259–R266. (https://doi.org/10.1530/ERC-19-0048)

42 Cho U, Mete O, Kim MH, Bae JS & Jung CK. Molecular correlates and rate of lymph node metastasis of non-invasive follicular thyroid neoplasm with papillary-like nuclear features and invasive follicular variant papillary thyroid carcinoma: the impact of rigid criteria to distinguish non-invasive follicular thyroid neoplasm with papillary-like nuclear features. *Modern Pathology* 2017 **30** 810–825. (https://doi.org/10.1038/modpathol.2017.9)

43 Rosario PW. Diagnostic criterion of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP): absence of papillae. *Human Pathology* 2019 **83** 225. (https://doi.org/10.1016/j.humpath.2018.05.029)

44 Yoon JH, Kim EK, Youk JH, Moon HJ & Kwak JY. Better understanding in the differentiation of thyroid follicular adenoma,

follicular carcinoma, and follicular variant of papillary carcinoma: a retrospective study. *International Journal of Endocrinology* 2014 **2014** 321595. (https://doi.org/10.1155/2014/321595)

45 Kim M, Jeon MJ, Oh HS, Park S, Kim TY, Shong YK, Kim WB, Kim K, Kim WG & Song DE. BRAF and RAS mutational status in noninvasive follicular thyroid neoplasm with papillary-like nuclear features and invasive subtype of encapsulated follicular variant of papillary thyroid carcinoma in Korea. *Thyroid* 2018 **28** 504–510. (https://doi.org/10.1089/thy.2017.0382)

46 Howitt BE, Paulson VA & Barletta JA. Absence of BRAF V600E in non-infiltrative, non-invasive follicular variant of papillary thyroid carcinoma. *Histopathology* 2015 **67** 579–582. (https://doi.org/10.1111/his.12680)

47 Borrelli N, Denaro M, Ugolini C, Poma AM, Miccoli M, Vitti P, Miccoli P & Basolo F. miRNA expression profiling of 'noninvasive follicular thyroid neoplasms with papillary-like nuclear features' compared with adenomas and infiltrative follicular variants of papillary thyroid carcinomas. *Modern Pathology* 2017 **30** 39–51. (https://doi.org/10.1038/modpathol.2016.157)

48 Giannini R, Ugolini C, Poma AM, Urpì M, Niccoli C, Elisei R, Chiarugi M, Vitti P, Miccoli P & Basolo F. Identification of two distinct molecular subtypes of non-invasive follicular neoplasm with papillary-like nuclear features by digital RNA counting. *Thyroid* 2017 **27** 1267–1276. (https://doi.org/10.1089/thy.2016.0605)

49 Kim TH, Lee M, Kwon AY, Choe JH, Kim JH, Kim JS, Hahn SY, Shin JH, Chung MK, Son YI, *et al*. Molecular genotyping of the non-invasive encapsulated follicular variant of papillary thyroid carcinoma. *Histopathology* 2018 **72** 648–661. (https://doi.org/10.1111/his.13401)

50 Najafian A, Noureldine S, Azar F, Atallah C, Trinh G, Schneider EB, Tufano RP & Zeiger MA. RAS mutations, and RET/PTC and PAX8/PPAR-gamma chromosomal rearrangements are also prevalent in benign thyroid lesions: implications thereof and a systematic review. *Thyroid* 2017 **27** 39–48. (https://doi.org/10.1089/thy.2016.0348)

Received in final form 18 October 2019
Accepted 31 October 2019
Accepted Preprint published online 31 October 2019