Abstract

The 2017 edition of the WHO book on Classification of Tumours of Endocrine Organs includes a new section entitled ‘Other encapsulated follicular-patterned thyroid tumours’, in which the newly created NIFTP (non-invasive follicular thyroid neoplasm with papillary-like nuclear features) is identified and described in detail. Despite deleting the word ‘carcinoma’ from its name, NIFTP is not a benign tumor either and is best regarded as a neoplasm with ‘very low malignant potential’. The main goal of the introduction of NIFTP category is to prevent overdiagnosis and overtreatment. Sampling constraints, especially when dealing with heterogeneous and/or large nodules, and difficulties in the invasiveness evaluation, are the major weaknesses of the histological characterization of NIFTP. At the cytological level, NIFTP can be separated from classic papillary carcinoma (cPTC) but not from encapsulated, invasive follicular variant PTC. The impact of NIFTP individualization for cytopathology is the drop of rates of malignancy for each Bethesda category in general and for indeterminate categories in particular. The biggest impact will be seen in institutions with a high frequency of FVPTC. The introduction of NIFTP has changed the utility of predictive values of molecular tests because \( RAS \) mutations and \( PAX8-PPARG \) rearrangements are frequently detected in NIFTP. This turns less promising the application of mutation detection panels as indicators of malignancy and will probably contribute to switch to a rule-out approach of molecular testing. Selection for surgery will go on being determined by a combined detection of clinical, cytological and ultrasound suspicious features.

Key Words
- thyroid cancer
- NIFTP
- cytology
- molecular tests
- risk of malignancy

Introduction

The fourth edition of the WHO book on Classification of Tumours of Endocrine Organs (Lloyd et al. 2017) includes a new section entitled ‘Other encapsulated follicular-patterned thyroid tumours’ in which, together with the two types of ‘UMPs’ (‘Follicular tumour of Uncertain Malignant Potential’ and ‘Well differentiated tumour of Uncertain Malignant Potential’), the newly created NIFTP (Non-invasive follicular thyroid neoplasm with
papillary-like nuclear features) is identified and described in detail (Cameselle-Teijeiro et al. 2017, Lloyd et al. 2017).

The name NIFTP is awkward – there is no such thing as papillary-like nuclear features – but it represents a compromise in order to avoid the word carcinoma and to protect patients from overdiagnosis and overtreatment of thyroid nodules (for a thorough discussion on these aspects see Nikiforov et al. (2016) and Xu et al. (2017)).

A similar attempt to avoid overdiagnosis and overtreatment had already been made in 2000 – Dillwyn Williams and the Chernobyl Group proposed the creation of two morphologic categories of thyroid tumors, the so-called UMPs, advancing that in such cases the most appropriate therapeutic attitude was ‘watchful waiting’ (Williams 2000). Despite the existence of similar approaches in other models (e.g. borderline and UMP tumors in several organs), many thyroid pathology experts considered that the utilization of the description ‘uncertain malignant potential’ was confusing for patients and clinicians. Due to these criticisms, the two categories of UMP tumors have not been adopted, namely in USA and Canada.

Although it is generally acknowledged that the encapsulated non-invasive form of the follicular variant of papillary thyroid carcinoma (E/NI-FVPTC) represents a neoplasm displaying very low malignancy that may be treated by conservative surgery (lobectomy or lobectomy plus isthmectomy) (Rosai 2010), this assumption was not accepted by many clinicians. The reasons advanced for such reluctance was the utilization of the word carcinoma in the diagnosis and the associated mandatory staging of such tumors as any other malignant neoplasm.

The aforementioned limitations have led to the creation of the NIFTP diagnostic category of thyroid neoplasms (Nikiforov et al. 2016) that represents a sort of pragmatic approach driven by the need to protect patients. This pragmatic approach contradicts the present trend toward precision medicine diagnosis in almost every oncological disorder. The situation is particularly vexing in thyroid carcinoma in general, and in PTC in particular, since the recently published The Cancer Genome Atlas (TCGA) has clarified the major molecular alterations in almost 97% of PTCs (Cancer Genome Atlas Research Network 2014).

In order to achieve the goal of the present review – to discuss and, whenever possible, elucidate the impact of the creation of NIFTP on the reclassification of thyroid nodules – we will start by reviewing, shortly, the history and the definition of the different types and subtypes of PTC that fall in the large group of FVPTC. Using both the data on record in the literature and our own experience, we will discuss afterwards the impact of the introduction of the NIFTP category in the histopathological classification of thyroid nodules. In the last section we will discuss, again using data from the literature and the results obtained in the revision of our cases, the impact on cytopathology of the creation of NIFTP.

**History of FVPTC and the recent creation of NIFTP**

The first classification of well-differentiated tumors of the thyroid was mainly based in the architectural patterns: nodular hyperplasia for non-encapsulated follicular-patterned lesions, follicular adenoma (FA) or follicular carcinoma (FTC) for encapsulated follicular-patterned lesions, the latter if capsular and/or vascular invasion was present, and papillary carcinomas for tumors displaying papillary formation. Later, Lindsay first (Lindsay 1960), and then Rosai afterwards (Chen & Rosai 1977), reported selected cases of tumors with pure or quasi-pure follicular growth pattern that exhibited similar behavior to ‘classic’ papillary carcinoma cPTC (more tendency to lymph node than to hematogenous metastasis) whenever the nuclear features were similar to those of tumors displaying papillary architecture. This led to the recognition of the follicular variant PTC (FVPTC). We know now that most of such FVPTC correspond to tumors we designate nowadays as invasive/infiltrative FVPTC (I-FVPTC), but that was not evident until recently. Since such nuclear features were typically present in PTC and distinguished them from other follicular-patterned lesions, the identification of PTC nuclear features became, per se, synonym of malignancy, regardless of the tumors being well circumscribed or infiltrative. As a consequence, invasiveness, a cardinal feature of malignancy in other organs, has become dispensable for diagnosing PTC for several decades.

Within the group of FVPTC, three clinicopathological entities were recognized: the encapsulated/well-circumscribed, non-invasive FVPTC (E/NI-FVPTC), with a more indolent behavior, the I-FVPTC, whose behavior is closer to cPTC and a third, much less frequent subtype of FVPTC, the multinodular/diffuse form of FVPTC. This multinodular FVPTC is characterized by an aggressive clinical behavior, giving rise to nodal and distant (lung and bone) metastases (Ivanova et al. 2002, Lloyd et al. 2017).

The crucial distinction between non-invasive and invasive forms of PTC has been reinforced by the finding...
of a molecular profile in E-FVPTC which is more similar to that of FA and FTC than to that of cPTC (Rivera et al. 2010, Ibrahim & Wu 2016, Cho et al. 2017). It is easy to separate the molecular profile of cPTC (‘BRAF V600E-like genotype’) in which there are frequent RET/PTC and NTRK rearrangements and less frequent RAS mutations, from the molecular profile of E-FVPTC (‘RAS-like genotype’) with frequent RAS mutations and PAX8-PPARγ rearrangements and rare molecular events of the other genotype (Rivera et al. 2010, Ibrahim & Wu 2016, Cho et al. 2017).

The same does not hold true regarding the I-FVPTC, which is not yet fully clarified, mainly because most series on record put together, within the category of FVPTC, encapsulated and infiltrative forms. The situation is even more complicated in the latter, since in this group (I-FVPTC), it is necessary to consider the existence of two subgroups of tumors: the bona fide I-FVPTC displaying a stellate architecture and, usually, interstitial fibrosis, and the I-FVPTC that appears to originate from a preexistent encapsulated/well-circumscribed FVPTC, which has acquired focal invasive properties at the periphery (a sort of E/I-FVPTC). We still ignore if the molecular features of I-FVPTC are different from those of E/I-FVPTC. It is plausible that the molecular profile of the former, which looks infiltrative/invasive from its inception, may be similar to cPTC, whereas the latter, that has apparently acquired invasive properties after being encapsulated, may be similar to E/NI-FVPTC (Cho et al. 2017).

The poor definition of minimal criteria for identifying PTC nuclear features has led to a progressive lowering of the threshold for diagnosing PTC. This trend gained its greatest expression in the fact that diagnoses of FA and FTC became gradually rarer and rarer... The identification of an ‘entity’ described as well-differentiated tumor of unknown malignant potential (WDT, UMP) that included cases with ‘insufficient’ PTC-like nuclear features, only partially ameliorated the problem of overdiagnosing malignancy (Williams 2000). The poor reproducibility inherent in the identification of nuclear features enough to diagnose ‘malignancy’ and the recognition that some forms of FVPTC (e.g. E/NI-FVPTC) carried a very indolent clinical course, raised concerns about the existence of many patients with a diagnosis of FVPTC being overtreated (Rosai 2010). It was clear that such patients did not benefit from total thyroidectomy–lobectomy alone and surveillance would be sufficient (Howitt et al. 2013, Thompson 2016a). It was also acknowledged the magnitude of the problem since FVPTC is the second most frequent variant of PTC (after microcarcinoma) and, among FVPTC, half to two-thirds of cases are E/NI-FVPTC (Lupi et al. 2007, Jung et al. 2014). It is now widely recognized that E/NI-FVPTC carries similar prognosis when treated by total thyroidectomy plus radioactive iodine or lobectomy alone (Asimakopoulous & Nixon 2017). That is the reason why, since 2009, the American Thyroid Association is recommending a conservative treatment for such cases, an attitude that clinicians have been reluctant to take in cases diagnosed as ‘carcinoma’ (American Thyroid Association Guidelines Taskforce 2009).

The concept and the designation NIFTP that encompasses former E/NI-FVPTC and WDT, UMP diagnosed due to equivocal PTC-like nuclear features, intend to constitute a way of solving the aforementioned problems through the utilization of terms (‘non-invasive’ and ‘tumor’ instead of ‘carcinoma’) that will induce a more conservative treatment. This option will reduce the iatrogeny, psycho-social burden and high economical costs for patients and medical care. Despite deleting the word ‘carcinoma’ from its name, NIFTP is not a benign tumor either and is best regarded as a neoplasm with ‘very low malignant potential’, since rare cases fulfilling its criteria may give rise to metastasis (Cho et al. 2017, Parente et al. 2017).

Strict inclusion and exclusion criteria are absolutely necessary to support the diagnosis of NIFTP (Nikiforov et al. 2016). Inclusion criteria are the following: follicular pattern, encapsulation/good circumscription and nuclear grade equal or above 2 (a nuclear grade score 0–3 was created to define minimal nuclear ‘atypia’ necessary to render a diagnosis of NIFTP instead of a diagnosis of follicular adenoma/adeno-matous nodule) (Nikiforov et al. 2016). Assuming the aforementioned entry criteria are present, the final diagnosis of NIFTP may be rendered unless any of the following exclusion features are present: vascular or capsular invasion, more than 1% papillae, presence of psamomma bodies, more than 30% solid/trabecular architecture, more than 3 mitoses/10 HPF and presence of tumor necrosis (Nikiforov et al. 2016).

Impact on histopathology

Strengths

The greatest advantages of the renaming/reclassification of some E/NI-FVPTC as NIFTP are the emphasis on the good prognostic value of expansive growth in thyroid neoplasms depicting papillary carcinoma-like nuclear features and the less-threatening nomenclature as a way to avoid overtreatment.
Weaknesses

Sampling constraints: The diagnosis of NIFTP can only be made in surgically removed specimens after total sampling of the lesions. At variance with the differential diagnosis of FA vs FTC, not only the capsule must be completely examined but also the center of the lesion. This may represent an extra problem for laboratory and medical workflow and will lead to high costs, especially when dealing with large nodules.

Reproducibility of the diagnosis

In our series, 31.6% of cases previously diagnosed, from 2005 to 2016, as FVPTC fulfilled the criteria of NIFTP (T Maia and I Amendoeira, unpublished observations). This percentage varies largely from series to series, ranging from 1.5% to 40.2% (Bychkov et al. 2017, Valderrabano et al. 2017, Yang et al. 2017). Since the majority of the studies on record were made in Western countries, different surveillance or different access to medical care are not expected to justify such a large variation, which is probably due to a heterogeneous application of diagnostic criteria.

The reproducibility of the diagnosis of NIFTP is unlikely to be attributable to differences in the evaluation of the nuclear features, since they have become less important than previously for diagnosing FVPTC. In our experience, the greatest diagnostic difficulties concern the identification of capsular and/or vascular invasion, the evaluation of the amount of solid/trabecular areas that need to be quantified as more or less than 30%, and the quantification of the focal papillae formation as more or less than 1%.

We (and others) are facing three additional diagnostic problems that may contribute to the different prevalence of NIFTP in the series on record: the issue of multinodular lesions in which one (or a few) lesion(s) display NIFTP features, the problem of oncocyic (or oncocyotoid) lesions (there is not, yet (?), an oncocyic NIFTP) and the issue of NIFTP-like lesions arising in FA with papillary hyperplasia (is it possible to make a diagnosis of NIFTP within benign larger lesions?). These three problems create difficulties in the diagnosis of NIFTP-like cases that do not entirely fit the published archetype. Some of these problems are addressed by LiVolsi and Baloch in their recent paper on NIFTP (LiVolsi & Baloch 2017).

Conceptual limitations

NIFTP is a poorly defined tumor entity that, in one side, has been carved out from WDT, UMP and, on the other, encompasses encapsulated and/or well-circumscribed, non-invasive FVPTC.

Most of the NIFTP diagnostic criteria are exclusion criteria, such as the percentage of papillary structures and of trabecular/solid areas that do not help to define an entity from a pathogenic or molecular standpoint (Nikiforov et al. 2016). The same holds true regarding the inclusion in the NIFTP group of cases in which nuclei do not display full-blown PTC features (score 2) or have nuclei displaying score 3 features in part of a tumor that displays mainly follicular cell nuclei (Nikiforov et al. 2016). This cellular/nuclear heterogeneity probably justifies the heterogeneity of the molecular profile of NIFTP (see below).

It is beyond the scope of the present review to discuss the putative mechanisms involved in the acquisition of invasive properties of the neoplastic cells. There is, however, evidence pointing to the role played by epigenetic alterations, namely miRNAs associated to the epithelial–mesenchymal transition of the neoplastic cells (Marini et al. 2011). More importantly, there is evidence highlighting the role played by vessels, fibroblasts and other stromal cells, as well as by extracellular matrix components of the periphery of the tumors, in the acquisition of invasive features (Eloy et al. 2011a, Montemayor-Garcia et al. 2013).

Molecular findings in NIFTP

Several studies comparing cPTC to E-FVPTC and FA/FTC have been made in the last years but the group of E-FVPTC still remains very heterogeneous. This heterogeneity reflects the difficulty in separating, in almost every series, NIFTP cases from FVPTC with invasion. In many series, it is not also possible to separate E-FVPTC with focal invasion (E/I-FVPTC) from I-FVPTC. A few recent studies have taken into account strict diagnostic criteria and studied the molecular features of NIFTP, aiming to find differences in the comparison of NIFTP with the following entities: E-FVPTC with invasion (E/I-FVPTC), I-FVPTC, cPTC and FA/FTC. These studies included not only genotype analyses (point mutations and gene fusion abnormalities), but also gene expression profiling (cPTC) and microRNA expression profiling (mRNA-EP) (Jiang et al. 2016, Cho et al. 2017).

Genotypic abnormalities reported to date are summarized in Table 1. The most frequent genetic abnormalities found in NIFTP are RAS (N-RAS, H-RAS or K-RAS) and BRAF mutations as well as PAX8-PPARγ rearrangements. As in FVPTC, such mutations appear to be mutually exclusive and were detected in a percentage
that varies between 44.4% and 100% of the cases. BRAF mutations are most frequently BRAFK601E, but BRAFV600E, initially thought to be absent in NIFTP, have also been reported in a minority of cases. So far, no RET/PTC rearrangement, typical of cPTC, has been described in cases of NIFTP.

Overall, the pattern of genetic abnormalities of NIFTP is closer to that of FA/FTC and to cases formerly classified as E-FVPTC, but there is also some overlap with cPTC.

The study from Cho and coworkers (Table 1) evaluated the impact of the threshold of acceptance of papillary architecture to diagnose NIFTP and found significant differences regarding prevalence of BRAFV600E mutations when comparing ‘NIFTP with no papillae at all’ vs ‘NIFTP with papillae’ (less than 1%) (Cho et al. 2017). No mutations were present in cases with no papillae formation, whereas the BRAFV600E mutation was detected in some cases of the other group. Although the morphologic demonstration of vascular invasion is a much better predictor of increased aggressiveness than the presence of BRAFV600E mutation per se (Eloy et al. 2011b), the aforementioned findings point to the need to

Table 1 Molecular abnormalities in NIFTP.

<table>
<thead>
<tr>
<th>Method</th>
<th>No of cases</th>
<th>Genes tested</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-mutated (%)</td>
</tr>
<tr>
<td>Nikiforov et al. (2016)</td>
<td>NGS</td>
<td>27</td>
<td>&gt;56 genes</td>
</tr>
<tr>
<td>Lee et al. (2017)</td>
<td>PCR</td>
<td>21</td>
<td>BRAF RAS RETisional NTRK1 ALK</td>
</tr>
<tr>
<td>Zhao et al. (2017)</td>
<td>NGS</td>
<td>48</td>
<td>21 genes</td>
</tr>
<tr>
<td>Jiang et al. (2016)</td>
<td>NGS</td>
<td>4</td>
<td>BRAF RAS RETisional PAX8/PPARG</td>
</tr>
<tr>
<td>Borrelli et al. (2017)</td>
<td>PCR</td>
<td>19</td>
<td>BRAF RAS RETisional PAX8/PPARG</td>
</tr>
<tr>
<td>Howitt et al. (2015)</td>
<td>PCR</td>
<td>9</td>
<td>BRAF RAS RETisional PAX8/PPARG</td>
</tr>
<tr>
<td>Bizarro et al. (2016)</td>
<td>PCR</td>
<td>13</td>
<td>BRAF RAS RETisional PAX8/PPARG</td>
</tr>
<tr>
<td>Ohori et al. (2017)</td>
<td>PCR</td>
<td>11</td>
<td>BRAF RAS RETisional PAX8/PPARG</td>
</tr>
<tr>
<td>Cho et al. (2017)</td>
<td>PCR</td>
<td>105 (Group 1) 95 (Group 2)</td>
<td>Group 1 BRAF RET/SV</td>
</tr>
<tr>
<td>Valderrabano et al. (2017)</td>
<td>NGS</td>
<td>5</td>
<td>&gt;56 genes</td>
</tr>
<tr>
<td>Giannini et al. (2017)</td>
<td>PCR</td>
<td>26</td>
<td>BRAF RAS</td>
</tr>
</tbody>
</table>

*Population with known high prevalence of BRAF V600E mutation.
NGS, next generation sequencing; PCR, polymerase chain reaction.
refine diagnostic criteria in order to try to obtain a more homogeneous molecular profile in NIFTP. The highest prevalence of \textit{BRAFV600E} mutations in NIFTP was found in a study performed in a Korean population (Lee et al. 2017), in which the overall prevalence of this mutation is high, raising the possibility that one may be dealing with a population-specific prevalence. Alternatively, such result may reflect less strict histological criteria used by local pathologists to diagnose NIFTP (namely including, as NIFTP, cases exhibiting few but more than 1% papillae) (Lee et al. 2017).

Since NIFTP may harbor, although rarely, \textit{BRAFV600E} mutations, one may conclude that this finding is not specific for I-FVPTC or cPTC and, hence, cannot be used \textit{per se} as a sign of clear cut malignancy. It was also already known that the \textit{BRAF V600E} mutation is rather frequent in microcPTC (Soares et al. 2004, Trovisco et al. 2005). Of note, a different mutation in the same gene, \textit{BRAF}K601E, is associated with NIFTP and E-FVPTC (Afkhami et al. 2016). This mutation has also been reported in I-FVPTC but not in cPTC (Rossi et al. 2015, Afkhami et al. 2016).

Besides the most frequent molecular abnormalities described earlier, others have been detected, namely TERT promoter (TERTp) mutations, which are associated, in other morphologic contexts, with worse prognosis (Melo et al. 2014, 2017). Whether NIFTP with TERTp mutations behaves in a similar way to NIFTP without TERTp mutation remains to be clarified.

Gene expression profiling (GEP) and mRNA-EP studies of NIFTP have been reported and two types of profiles were found: a profile similar to I-FVPTC and a profile similar to FA (Denaro et al. 2017, Giannini et al. 2017). In the study by Giannini and coworkers, two clusters of GEP were identified and found to be correlated with the underlying genetic abnormalities: an adenoma-like GEP that was associated with a wild-type profile and an I-FVPTC-like GEP associated with the presence of \textit{RAS} or \textit{BRAF} mutation (Giannini et al. 2017). NIFTP cases were equally distributed between the two clusters (Giannini et al. 2017). Immunohistochemical expression of Galectin-3 and HBME-1, known to be detected in some cases of FVPTC, is not able to distinguish NIFTP from I-FVPTC since both antigens may also be expressed in the former (Thompson 2016a). Together with morphology, molecular data also illustrate the existence of a great overlap between NIFTP and I-FVPTC leading to the conclusion that molecular pathology cannot be reliably used as a gold standard for solving problems of diagnostic subjectivity. What drives invasive growth from a preexisting non-invasive tumor remains unclarified and may be related with the stromal response of the host (see above).

Finally, a recent study from Fu et al. (2017) used programmed cell death ligand 1 (PD-L1) immunohistochemical expression to support a diagnosis of E/I-FVPTC over NIFTP in doubtful cases and found that the expression score was significantly higher in E/I-FVPTC than in NIFTP. The expression score of NIFTP was similar to that of benign nodules, suggesting that PD-L1 may be used as a differential diagnostic tool (Fu et al. 2017). These findings are consistent with previous studies associating PD-L1 expression with more aggressive behavior in thyroid cancer but their putative diagnostic utility has to be confirmed in other series (Chintakuntlawar et al. 2017).

Impact on cytopathology

Since Chen & Rosai (1977) described, in 1997, six tumors with a follicular growth pattern that displayed nuclear features and biological behavior similar to those of PTCs and designated them as FVPTC, cytopathologists became very attentive to nuclear features (enlargement, crowding, grooves, chromatin clearing, marginally placed micronucleoli and pseudoinclusions), whenever facing a follicular-patterned thyroid tumor.

Cytological specimens of thyroid lesions started being classified according to The Bethesda System for Reporting Thyroid Cytopathology (TBSTC) in 2009 (Cibas et al. 2009). The system encompasses six diagnostic categories (I. Non-diagnostic; II. Benign; III. Atypia of undetermined significance/Follicular lesion of undetermined significance (AUS/FLUS); IV. Suspicious for follicular neoplasm/Follicular neoplasm (SN/FN); V. Suspicious for malignancy (SM); VI. Malignant). For each category, the risk of malignancy has been stratified and a specific management has been recommended (Cibas et al. 2009).

Cytology is unable to establish a firm differential diagnosis between NIFTP, E/NI-FVPTC and I-FVPTC as these diagnoses depend on the demonstration of invasion, either capsular or vascular. Despite this, some authors (Howitt et al. 2015, Bizzarro et al. 2016, Ibrahim & Wu 2016, Maletta et al. 2016, Strickland et al. 2016) compared the cyto-architectural features of cPTC, FA, NIFTP and I-FVPTC in FNA, trying to find criteria able to provide a preoperative diagnosis (Brandler et al. 2017) (Table 2).

The results obtained to date demonstrate that NIFTP can be separated from cPTC by the presence of multiple nuclear pseudoinclusions, papillae and nuclear clearing in the latter. The presence of microfollicles and PTC nuclear features should trigger the possibility of NIFTP.
As far as the differential diagnosis between NIFTP and encapsulated, invasive FVPTC (E/I-FVPTC) is concerned, Maletta et al. (2016) found that NIFTP cannot be separated from E/I-FVPTC at the cytological level, and Bizarro et al. (2016) corroborated these findings, stressing nevertheless that a larger nuclear size and the presence of nuclear grooves favor the diagnosis of FVPTC. For Ibrahim & Wu (2016), NIFTP can be distinguished from I-FVPTC based on the magnitude of morphologic features and the demonstration that NIFTP falls commonly in the category of AUS/FLUS (61% of the cases), whereas I-FVPTC is frequently diagnosed at cytology as SM (44% of the cases) but the aforementioned differences are not that impressive.

Overall, the evaluation of NIFTP lesions and I-FVPTC by FNA has proven to be very difficult with a 29% false-positive rate and 9–58% true predictive value (Thompson 2016b).

Taking all this into consideration, as well as our own experience, it may be anticipated that the cytology smears of AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; DD, differential diagnosis; E/I-FVPTC, encapsulated invasive FVPTC; FA, follicular adenoma; FN, follicular neoplasm; FNA, fine-needle aspiration; I-FVPTC, invasive FVPTC; NIFTP, noninvase follicular tumor with papillary-like nuclear features; ROM, risk of malignancy; SM, suspicious for malignancy; TBRSTC, the Bethesda reporting system for thyroid cytology.

### Table 2
Summary of the most important data reported in studies addressing cytomorphologic features of NIFTP in pre-surgical FNA cytology (Brandler et al. 2017).

<table>
<thead>
<tr>
<th>Study aim</th>
<th>Cytomorphologic features</th>
<th>Most frequent TBRSTC for NIFTP cases</th>
<th>Results</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strickland et al. (2016)</td>
<td>Microfollicular architecture, papillae, psammomatous calcifications, sheet-like architecture, nuclear pseudooinclusions</td>
<td>SM</td>
<td>62% NIFTP 12.5% FVPTC 25% FA</td>
<td>cPTC can be separated from NIFTP/FVPTC on FNA</td>
</tr>
<tr>
<td>Howitt et al. (2015)</td>
<td>Microfollicular vs sheet, papillae, pseudooinclusions</td>
<td>SM</td>
<td>cPTC: 96% sheets 79% pseudooinclusions 50% papillae 4% microfollicles NIFTP: 0% papillae 0% pseudo inclusions 36% sheets 55% microfollicles</td>
<td>Cytology and molecular features can help to identify NIFTP at the time of diagnosis</td>
</tr>
<tr>
<td>Ibrahim &amp; Wu (2016)</td>
<td>Cellularity, nuclear irregularity, grooves, pseudooinclusions, nuclear enlargement</td>
<td>AUS/FLUS</td>
<td>NIFTP diagnosed as AUS/FLUS (61%) FVPTC diagnosed as SM (44%)</td>
<td>Cytology can distinguish between NIFTP and IFVPTC</td>
</tr>
<tr>
<td>Maletta et al. (2016)</td>
<td>Nuclear enlargement, nuclear abnormalities, microfollicular pattern, colloid, prominent nucleoli, tridimensional clusters</td>
<td>FN</td>
<td>Nuclear features with good correlation with histology The NIFTP nuclear features different from those of benign nodules but not from E/I-FVPTC Larger nuclear size and grooves more frequent in FVPTC</td>
<td>NIFTP and IFVPTC can not be reliably separated</td>
</tr>
<tr>
<td>Bizarro et al. (2016)</td>
<td>Colloid, papillae, isolated cells, N/C ratio, follicular architecture, pseudo-inclusions, grooves</td>
<td>AUS/FLUS</td>
<td>NIFTP usually lack pseudo-inclusions and papillary structures NIFTP and IFVPTC may be discriminated on cytology, based on nuclear size and microfollicular clusters</td>
<td></td>
</tr>
</tbody>
</table>
of NIFTP will be usually hypercellular, mainly made up of microfollicles, and ‘bare’ enlarged nuclei. This means that a cytological diagnosis of, at least, ‘follicular tumor’ (Bethesda IV) should be made whenever facing a smear from a NIFTP. Besides the aforementioned features, nuclear crowding, clear chromatin and nuclear grooves, as seen in cPTC, may be present as well. Nuclear pseudo-inclusions are not a feature of NIFTP, nor are psammomatous microcalcifications, both being much more frequent in cPTC. Whenever numerous pseudo-inclusions are observed in a thyroid FNA one should search for other features of cPTC (papillae, overlapped elongated nuclei, metaplastic cytoplasm).

The majority of NIFTP cases are classified in the Bethesda ‘Indeterminate’ categories (Table 3) – atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), suspicious for follicular neoplasm/follicular neoplasm (SFN/FN) or even suspicious for malignancy (SM) (Strickland et al. 2015, Faquin et al. 2016, Maletta et al. 2016). Taking into consideration the morphological and molecular heterogeneity of NIFTP, it is not surprising the spreading of cytological diagnosis of NIFTP through different Bethesda categories, depending on the assessment of nuclear atypia and on institutional recommendations.

Some groups have already proposed modifications for the next Bethesda edition advancing that the smears from cases of NIFTP should fall in the ‘SFN/FN’ category (Pusztaszeri et al. 2016).

As a consequence of the spread of cytological diagnoses and absence of strict cytological criteria, the risk of malignancy (ROM) for each category is changing with the introduction of NIFTP (not considered a clinically malignant tumor) in routine diagnosis (Table 4). The decrease of the risk of malignancy (ROM) varies from series to series and is reported to be from 13.5% to 48% in the SM category, 10–36% in the SFN/FN and 4.9–45% in the AUS/FLUS category (Table 4). For the Bethesda VI (malignant) category, the decrease of ROM secondary to the introduction of NIFTP is not relevant. The magnitude of the impact varies from series to series, the bigger impacts being seen in institutions with higher frequency of cases that used to be diagnosed as FVPTC.

To improve preoperative diagnosis, efforts have been put in molecular testing, both ‘rule-in’ and ‘rule-out’ tests using the molecular information on record (Howitt et al., 2013, Beaudenon-Huibregtse et al., 2014, Labourier et al. 2016, Wong et al., 2016, Eszlinger et al., 2017, Lee et al., 2017, Valderrabano et al. 2017). Unfortunately, seventy per cent of the cases studied in The Cancer Genome Atlas (TGCA) focused on cPTC (Cancer Genome Atlas Research Network 2014) and only a minority focused on FVPTC (Basolo et al. 2017).

The rule-out test (Afirma GEC) aims to identify benign nodules and can be used in the ‘Indeterminate’ FNA cytology; the samples are classified as ‘benign’ or ‘suspicious’. It is important to consider that the pre-test ROM (prevalence of malignancy in the local test setting)
The finding of a RAS mutation or a PAX8-PPARγ rearrangement (Nikiforova et al. 2002, Cheung et al. 2003) in histologically benign nodules is a major limitation to mutation detection panels (Beaudenon-Huibregtse et al. 2014, Eszlinger et al. 2017) and has turned unacceptable the statement that finding a RAS mutation or a PAX8-PPARγ rearrangement in a cytological smear was an indicator of malignancy (Nikiforov et al. 2016). However, some authors claim that the detection of such genetic alterations might be used as an indicator of caution in histopathologic evaluation (Cibas et al. 2009). On the other hand, some unexpected negative molecular results, considered by some as ‘false-negative’ results, belong to NIFTP (Beaudenon-Huibregtse et al. 2014).

The reduction in the ROM in the indeterminate categories conditioned by the introduction of NIFTP will probably switch the molecular diagnosis to a rule-out approach (Eszlinger et al. 2017). This switch reflects of the increase of the NPV and the absence of clinical significance in terms of malignancy of the detection of several genetic structural alterations (see above).

According to the Guidelines of the National Comprehensive Cancer Network (NCCN) a post-test ROM of ≤5% is required to opt for watchful waiting instead of diagnostic surgery (Tuttle et al. 2010).

It is crucial that any molecular FNA diagnostic approach is considered along with a high-quality cytopathology diagnosis and that each institution establishes its own malignancy rates for cytology, taking also in consideration the ultrasound risk evaluation.

### Table 5 ‘Ruling-out’ malignancy according to Bethesda indeterminate categories.

<table>
<thead>
<tr>
<th>Rule-out Afirma GEC</th>
<th>AUS/FLUS</th>
<th>SFN/FN</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexander et al. (2012), Eszlinger et al. (2017)</td>
<td>NPV 95%</td>
<td>NPV 94%</td>
<td>NPV 85%</td>
</tr>
</tbody>
</table>

AUS/FLUS, atypia of unknown significance/follicular lesion of unknown significance; NPV, negative predictive value; PPV, positive predictive value; SFN/FN, suspicious for follicular neoplasm/follicular neoplasm; SM, suspicious of malignancy.

influences test reliability. For the Afirma GEC, a pre-test ROM lower than 23% for AUS/FLUS cytology is required to achieve a negative predictive value (NPV) higher than 95% and a post-test ROM lower than 5% (Eszlinger et al. 2017). It is calculated that for an institution with a ROM of 48%, the NPV of Afirma GEC will drop to 85% (Eszlinger et al. 2017) (Table 5). The ROM for each cytological category has to be determined for each local setting before the Afirma GEC test is used.

As far as rule-in tests are concerned, the observed different sensitivity value and positive predictive value (PPV) (Table 6) can be explained by the inter- and intra-observer variability both in cytological and histological interpretation, as well as by the different percentages of cPTC, FVPTC and FTC with their own different mutation frequencies. It also depends on the ‘local’ malignancy rates and the referral practice of each center.

The introduction of NIFTP concept has changed the utility of predictive values of molecular tests (Strickland et al. 2015, Faquin et al. 2016); Wong and coworkers reported that NIFTP accounted for 64% of the Afirma GEC (Wong et al. 2016); Valderrabano et al. (2017) retrospectively reassessed NIFTP with previous categories AUS/FLUS and SFN/FN and found a drop on ROM from 24% to 15%, an increase of NPV from 88% to 94% and a decrease of PPV from 50% to 34% (9).

The institutional frequency of diagnosing encapsulated FVPTC vs NIFTP will determine the variation of the predictive value of molecular tests (Baloch et al. 2016).

### Table 6 ‘Ruling-in’ malignancy (NGS) according to Bethesda indeterminate categories.

<table>
<thead>
<tr>
<th>Nikiforov et al. (2016)</th>
<th>AUS/FLUS</th>
<th>SFN/FN</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaudenon-Huibregtse et al. (2014)</td>
<td>PPV 88%</td>
<td>PPV 87%</td>
<td></td>
</tr>
<tr>
<td>Labourier et al. (2016)</td>
<td>Sensitivity 63%</td>
<td>Sensitivity 57%</td>
<td></td>
</tr>
<tr>
<td>Eszlinger et al. (2017)</td>
<td>Specificity 99%</td>
<td>Specificity 69%</td>
<td></td>
</tr>
</tbody>
</table>

AUS/FLUS, atypia of unknown significance/follicular lesion of unknown significance; PPV, positive predictive value; SFN/FN, suspicious for follicular neoplasm/follicular neoplasm; SM, suspicious of malignancy.
The major advantage of NIFTP for cytology is the reduction of SM category (suspicious for malignancy – Bethesda V) diagnosis which, in follicular-patterned tumors, is very much dependent on good quality smears and on the cytopathologist expertise. The main disadvantage is the ‘false-negative’ diagnosis in the SFN/FN category (Bethesda IV) leading to the need of a second surgery for totalization of thyroidectomy in cases of I-FVPTC.

In conclusion, we agree with Strickland et al. (2015) regarding the main impacts of NIFTP for cytopathology that are summarized in Table 7.

Table 7  Main impacts of NIFTP on cytopathology.

1. Drop of rates of malignancy for each Bethesda category
2. Striking relative decrease of risk of malignancy for atypia of unknown significance/follicular lesion of unknown significance (4.9–45%), suspicious of follicular neoplasm/follicular neoplasm (10–36%) and SM (13–48%)
3. Very low impact of risk of malignancy in the malignant category (1–5%) – most nodules still keep the diagnosis of malignancy
4. The biggest impact will be seen in institutions where the rate of FVPTC is highest
5. Molecular testing will probably switch to rule-out approach
6. Selection for surgery will be mostly determined by clinical, cytological and ultrasound suspicious features

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**Declaration of interest**

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

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