ABSTRACT

Objectives: Endocrine pathologists are reconsidering whether tumors characterized as noninvasive follicular variant of papillary thyroid carcinoma (NFVPTC) warrant a diagnosis of carcinoma. A change in terminology would affect cytology diagnoses; thus, our aim was to study the preceding fine-needle aspiration (FNA) diagnoses of this group of tumors.

Methods: We evaluated the FNA diagnoses of a primary cohort of 72 consecutively resected NFVPTCs and the cytologic and molecular features of an additional cohort of 39 tumors that included both NFVPTCs and classical papillary thyroid carcinomas (cPTCs).

Results: For our primary cohort, the preceding FNA diagnosis associated with the highest risk of malignancy was suspicious for PTC in nearly half (48.6%) of cases. In contrast to the majority of cPTCs, no NFVPTCs in our second cohort had papillae or pseudoinclusions on cytologic evaluation of the FNA specimens, and none harbored a BRAF V600E mutation.

Conclusions: If NFVPTCs were no longer termed carcinomas, this would affect the rate of malignancy of FNA diagnostic categories. Cytologic and molecular features could aid in identifying NFVPTCs at the time of FNA diagnosis.

While the incidence of thyroid carcinoma has nearly tripled in the past three decades, there has been essentially no change in mortality, indicating that many of these tumors are indolent and that overtreatment is occurring. In the past decade, the indolent nature of many follicular variant of papillary thyroid carcinomas (FVPTCs) has been increasingly recognized, and it has become clear that not all FVPTCs are prognostically or molecularly alike. FVPTCs with an infiltrative growth pattern are associated with frequent lymph node metastases, a risk of recurrence, and a BRAF mutation frequency of roughly 25%. In contrast, several studies have shown that encapsulated or partially encapsulated/well-circumscribed FVPTCs with no associated capsular penetration or lymphovascular invasion (ie, noninvasive FVPTCs [NFVPTCs]) have virtually no metastatic potential or risk of recurrence. Addition, while there is some variability in results (with Lee et al reporting no difference in molecular alterations between NFVPTCs and infiltrative FVPTCs), our group and others have found that NFVPTCs harbor RAS mutations and PAX8/PPARG rearrangements but lack BRAF mutations. Thus, insight into the prognostic and molecular findings of these tumors, along with increased awareness regarding the overtreatment of thyroid cancer, has prompted endocrine pathologists to reconsider whether NFVPTC warrants a diagnosis of carcinoma.

If the terminology of these tumors changes, this will affect the rate of malignancy associated with fine-needle aspiration (FNA) diagnostic categories. The 2007 National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference and the subsequent Bethesda System for Reporting Thyroid Cytopathology put forth a classification scheme for thyroid FNA diagnoses.
tumors had a complete capsule or partial capsule, whereas
of PTC. The tumors had a variably present capsule. Some
(ie, tumor to be diagnosed as FVPTC, the tumor was required
Based on the slide review, NFVPTCs were identified. For a
(B.E.H. and J.A.B.) blinded to the preceding FNA diagnoses.

between August 2010 and May 2012. All available tumor
specimens with FVPTC measuring 1 cm or more diagnosed
Women's Hospital (BWH) was performed for resection
Case Selection and Slide Review for Primary Cohort
Materials and Methods

Case Selection and Slide Review for Primary Cohort

A search of the pathology database at Brigham and
Women's Hospital (BWH) was performed for resection
specimens with FVPTC measuring 1 cm or more diagnosed
between August 2010 and May 2012. All available tumor
slides for each case were reviewed by two pathologists
(B.E.H. and J.A.B.) blinded to the preceding FNA diagnoses.
Based on the slide review, NFVPTCs were identified. For a
tumor to be diagnosed as FVPTC, the tumor was required
to have an entirely or almost entirely follicular architecture
(ie, ≤1% papillary architecture) along with nuclear features
of PTC. The tumors had a variably present capsule. Some
tumors had a complete capsule or partial capsule, whereas
others lacked a capsule altogether. However, in all cases,
the tumor was well circumscribed and lacked areas of tumor
infiltrating into benign thyroid parenchyma. Cases with any
indication of an infiltrative edge were considered infiltrative
and excluded. Encapsulated tumors with either lymphovas-
cular invasion (present within the capsule or beyond) or
complete capsular penetration were categorized as invasive
and were also excluded from this study. Cases with an ana-
plastic or a poorly differentiated component were excluded
(with a poorly differentiated component defined according
to the criteria described in the Turin proposal).23 After these
inclusion and exclusion criteria were met, we analyzed a
total of 72 consecutive cases of NFVPTC. Data extract-
ed from original pathology reports or electronic medical
records included age at diagnosis, sex, size of tumor (cm),
and presence of lymph node or distant metastases at the
time of the initial resection or completion thyroidectomy.

Preceding FNA Diagnoses of Primary Cohort

For each case in our cohort, the preceding cytology
diagnoses were recorded. FNAs that were performed at
BWH were done using a 25-gauge needle by an endocri-
nologist under ultrasound guidance (typically three or four
passes). The specimen was collected immediately in Cyto-
Lyt (Hologic, Marlborough, MA), and a single Papanico-
laou-stained ThinPrep slide was prepared using the ThinPrep
2000 (Hologic). The FNA diagnoses were recorded from the
pathology reports generated by staff cytopathologists using
a six-tiered diagnostic system essentially identical to that
eucidated in the 2007 National Cancer Institute Thyroid
Fine Needle Aspiration State of the Science Conference and
the subsequent Bethesda System for Reporting of Thyroid
Cytopathology.17-19 The diagnostic categories were ND,
benign, AUS/FLUS, FOL, SUS, and malignant (positive
for PTC). All FNA diagnoses corresponding to each tumor
were recorded (cytology diagnoses corresponding to differ-
ent nodules were not recorded). The preceding cytologic
diagnosis associated with the highest risk of malignancy
was identified for each case.

Assessment of Cytologic Features of NFVPTC
and cPTC in a Secondary Cohort

To investigate whether cases of NFVPTC can be distin-
guished from cPTC at the time of FNA diagnosis, we evalu-
ated an additional cohort (partially overlapping with the
previously described primary cohort) of 39 cases comprising
NFVPTCs and cPTCs. All of these cases had a preceding
cytology diagnosis of SUS or malignant and sufficient FNA
material for molecular analysis. This cohort included 11
(28%) NFVPTCs and 28 (72%) cPTCs. For each case, the
preceding cytology slides were reviewed by three patholo-
gists (S.C., J.F.K., and J.A.B.) at a multiheaded microscope
for the presence of the following features: a predominance of

Materials and Methods

scheme includes six diagnostic categories: nondiagnostic
(ND), benign, atypia/follicular lesion of undetermined sig-
nificance (AUS/FLUS), suspicious for follicular/Hürthle
cell neoplasm (FOL), suspicious for malignancy (SUS), and
malignant. One important objective of the Bethesda system
was to link a risk of malignancy with each diagnostic catego-
ry. Based on review of prior literature, the risk of malignan-
cy was cited to be 1% to 4% for ND, 0% to 3% for benign,
5% to 15% for AUS/FLUS, 15% to 30% for FOL, 60% to
75% for SUS, and 97% to 99% for malignant.19 Because of
the importance of this linked risk, subsequent studies further
evaluated the risk of malignancy of the most controversial
Bethesda category, AUS/FLUS. VanderLaan et al20 and
Ho et al21 both demonstrated a risk of roughly 25% in their
cohorts, a rate of malignancy significantly higher than that
first put forth for AUS/FLUS and a rate closer to that of the
FOL category.22 As a result, VanderLaan et al20 questioned
whether nodules with an AUS/FLUS FNA diagnosis should
go straight to lobectomy (the indicated procedure for a FOL
diagnosis) rather than undergoing additional assessment with
a repeat FNA. Hence, this example illustrates that a change
in the risk of malignancy for a Bethesda category not only
affects patient counseling but may also cast doubt on cur-
rent treatment algorithms. The purpose of this study was to
determine the preceding FNA diagnoses of NFVPTC to start
to collect information on the impact that a change in termin-
ology of these tumors may have on the risk of malignancy
for each FNA diagnostic category. In addition, we evaluated
the FNA specimens of a smaller cohort of NFVPTCs and
classical papillary thyroid carcinomas (cPTCs) for cytologic
features and molecular alterations in

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microfollicles vs a predominance of tumor sheets, presence of papillae, and presence of nuclear pseudoinclusions.

Assessment of Molecular Alterations in NFVPTC and cPTC in the Secondary Cohort

Molecular analysis for BRAF, NRAS, HRAS, and KRAS point mutations as well as RET/PTC and PAX8/PPARG rearrangements were performed on the remaining FNA material (suspended in Cytolyt). The original ThinPrep vial was stored at room temperature at BWH before being transported to the University of Leipzig and stored at 8°C for up to 6 months prior to the extraction of DNA and RNA. In brief, the remaining material (suspended in Cytolyt) was centrifuged for 5 minutes at 6,000 g, and then the pellet was used for DNA and RNA extraction as described previously. 24,25 Point mutations were detected by high-resolution melting polymerase chain reaction (PCR) and pyrosequencing as described previously. 25 Rearrangements were detected by quantitative PCR as described previously. 24

Statistical Analysis

Correlations were examined using the Fisher exact test. P values less than .05 were considered significant. Statistical analysis was performed using GraphPad InStat (GraphPad Software, La Jolla, CA).

Results

Clinical and Pathologic Features of the Primary Cohort

Our primary cohort of NFVPTCs included 72 tumors from 72 patients, including 56 (78%) women and 16 (22%) men. The mean age at the time of thyroidectomy was 53 years (range, 29-82 years), with 23 (32%) younger than 45 years and 49 (68%) 45 years or older. The mean tumor size was 2.5 cm (range, 1-6.5 cm). Forty (55.5%) were 1 to 2 cm (pT1), 22 (30.5%) were larger than 2 to 4 cm (pT2), and 10 (14%) were larger than 4 cm (pT3). By definition, no tumors had associated lymphovascular invasion or extrathyroidal extension. Lymph nodes were resected in 33 (46%) cases either at the time of the initial resection or at the time of completion thyroidectomy. The mean number of lymph nodes resected was 2.3 (range, 1-12). Only one patient had lymph node metastases. The one case with positive lymph nodes also harbored a separate 3.5-cm-tall cell variant of PTC, and the lymph node metastases had a papillary architecture with areas of tall cell morphology. No cases had distant metastases.

Preceding FNA Diagnoses of the Primary Cohort

Preceding FNA diagnoses were available for all cases. Fifty-one (71%) had one preceding FNA, 20 (28%) had two preceding FNAs, and one (1%) had three preceding FNAs. The preceding FNA diagnosis associated with the highest risk of malignancy was malignant (positive for PTC) in five (6.9%), SUS in 35 (48.6%), FOL in seven (9.7%), AUS/FLUS in 13 (18%), benign in nine (12.5%), and ND in three (4.2%). For the cases with two preceding FNAs, initial FNA diagnoses were as follows: ND in four (20%), benign in four (20%), AUS/FLUS in 10 (50%), and FOL in two (10%). On the subsequent FNA, a cytologic diagnosis was rendered with the same or lower associated risk of malignancy for 10 (50%) cases and a higher risk of malignancy for 10 (50%) cases. In the one case with three preceding FNAs, the first FNA was ND, followed by two AUS/FLUS FNAs. Examples of paired cytology and surgical resections are shown in Image 11 and Image 21.

Cytologic Features of NFVPTC Compared With cPTC in the Secondary Cohort

The results of our additional cohort of 39 PTC cases (11 NFVPTCs, 28 cPTCs) that had a SUS or malignant diagnosis on FNA and had undergone molecular analysis for BRAF, RAS, PAX8/PPARG, and RET/PTC alterations are summarized in Table 1. Of the 28 cPTCs, 22 (79%) were classified as malignant on preceding FNA, and six (21%) were diagnosed as SUS. In contrast, all 11 of the NFVPTCs in this cohort were diagnosed as SUS. cPTCs were significantly more frequently associated with a predominance of tumor sheets, papillae, and pseudoinclusions compared with NFVPTCs (P values of .0002, .0030, and <.0001, respectively). cPTCs had a predominance of tumor sheets in 27 (96%) cases, had papillae present in 14 (50%) cases, and pseudoinclusions identified in 22 (79%) cases. In contrast, of the NFVPTCs, four (36%) cases had a predominance of tumor sheets, and no cases had papillae or pseudoinclusions. Only one (4%) cPTC demonstrated a predominance of microfollicles compared with six (55%) cases of NFVPTC (P = .0009). One (9%) NFVPTC demonstrated a mixed population of microfollicles and tumor sheets, with neither architecture predominating. Examples of cases with the cytologic features assessed on review of the FNA specimens are shown in Image 31.

Molecular Features of NFVPTC Compared With cPTC in the Secondary Cohort

Molecular analysis was successful in 32 (82%) of these 39 cases. Of the 23 cPTCs with molecular analysis results, 14 (61%) harbored a BRAF V600E mutation, one (4%) had an NRAS mutation, and eight (35%) lacked a BRAF, RAS, PAX8/PPARG, or RET/PTC alteration. Of the nine NFVPTCs with molecular analysis, three (33%) had a RAS mutation (one NRAS, one HRAS, and one KRAS mutation), one (11%) had a PAX8/PPARG rearrangement, and...
five (56%) lacked a BRAF, RAS, PAX8/PPARG, or RET/PTC alteration. No case of NFVPTC contained a BRAF V600E mutation.

Discussion

In the setting of a heightened awareness of the overtreatment of thyroid carcinoma, the increased recognition of the indolent nature of NFVPTCs along with greater insight into the molecular alterations of these tumors has prompted endocrine pathologists to reconsider whether NFVPTCs warrant a diagnosis of carcinoma. However, a change in terminology would significantly affect the rate of malignancy of FNA diagnostic categories. This is especially true given the finding that over the past three decades, FVPTCs are accounting for a greater percentage of PTCs overall. For example, in a study by Jung et al, a follicular growth pattern was seen in 18% of PTCs diagnosed between 1974 and 1985 and in 57% of cases diagnosed in 2009. The purpose of this study was to determine the preceding FNA diagnoses of NFVPTC to start to collect information on the impact that a change in terminology of these tumors may have on the risk of malignancy for each FNA diagnostic category.
Our primary cohort included 72 tumors from 72 patients. Using the Bethesda System for Reporting of Thyroid Cytopathology, the preceding FNA diagnosis associated with the highest risk of malignancy was malignant (positive for PTC) in five (6.9%), SUS in 35 (48.6%), FOL in seven (9.7%), AUS/FLUS in 13 (18%), benign in nine (12.5%), and ND in three (4.2%). The low percentage of cases with a malignant diagnosis on FNA is consistent with what has previously been reported in the literature for FVPTC and is generally explained by the fact that FVPTC on FNA, just as on the resection, lacks papillae and is composed of cells with nuclear features that are often more subtle and focal.

Our primary cohort included 72 tumors from 72 patients. Using the Bethesda System for Reporting of Thyroid Cytopathology, the preceding FNA diagnosis associated with the highest risk of malignancy was malignant (positive for PTC) in five (6.9%), SUS in 35 (48.6%), FOL in seven (9.7%), AUS/FLUS in 13 (18%), benign in nine (12.5%), and ND in three (4.2%). The low percentage of cases with a malignant diagnosis on FNA is consistent with what has previously been reported in the literature for FVPTC and is generally explained by the fact that FVPTC on FNA, just as on the resection, lacks papillae and is composed of cells with nuclear features that are often more subtle and focal.

**Table 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>cPTC, No. (%)</th>
<th>NFVPTC, No. (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious on FNA</td>
<td>6 (21)</td>
<td>11 (100)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Malignant on FNA</td>
<td>22 (79)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Microfollicle predominant</td>
<td>1 (4)</td>
<td>6 (55)</td>
<td>.0009</td>
</tr>
<tr>
<td>Sheet predominant</td>
<td>27 (96)</td>
<td>4 (36)</td>
<td>.0002</td>
</tr>
<tr>
<td>Papillae</td>
<td>14 (50)</td>
<td>0</td>
<td>.0030</td>
</tr>
<tr>
<td>Pseudoinclusions</td>
<td>22 (79)</td>
<td>0</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>14 (61)</td>
<td>0</td>
<td>.0018</td>
</tr>
</tbody>
</table>

cPTC, classical papillary thyroid carcinoma; FNA, fine-needle aspiration; NFVPTC, noninvasive follicular variant of papillary thyroid carcinoma.

Molecular analysis was available for 23 cPTCs and nine NFVPTCs. One case of NFVPTC showed a mixed population of tumor sheets and microfollicles.
As a result, FVPTCs are often associated with a previous diagnosis of AUS/FLUS or even FOL on FNA. Thus, while the low rate of a malignant diagnosis on FNA and the frequent preceding AUS/FLUS and FOL diagnoses did not come as a surprise, the high percentage of cases with a SUS diagnosis was an unexpected finding. That said, this finding is not necessarily inconsistent with prior studies. Ustun et al found that 38% of their FVPTCs were diagnosed as either positive for PTC or SUS; however, this cohort cannot be directly compared with ours since it included all FVPTCs and not just NFVPTCs, which are often associated with more subtle nuclear features than infiltrative FVPTCs. In the one study that is closest to our current study, Lee et al evaluated the preceding FNA diagnoses of 30 encapsulated FVPTCs (this group likely also included partially encapsulated/well-circumscribed tumors) and found that the preceding FNA diagnosis was benign for 5% of cases, AUS/FLUS for 19% of cases, FOL for 14% of cases, SUS for 29% of cases, and positive for PTC in 34% of cases. The high rate of positive for PTC in their study compared with ours seems likely to be related to the fact that 23% of encapsulated FVPTCs in their cohort harbored BRAF mutations. This high rate of BRAF mutations is in contrast to previous findings reported by our group and those of Rivera et al. The reason for this different molecular profile is not entirely clear but potentially could be explained by the fact that the cohort of Lee et al included subcentimeter tumors, whereas our cohort and the cohort reported by Rivera et al did not. It is possible that for these smaller PTCs, a papillary architecture or infiltrative growth pattern

**Image 31** Features assessed on fine-needle aspiration (FNA) samples (Papanicolaou). **A**, Microfollicles in a noninvasive follicular variant of papillary thyroid carcinoma (PTC). **B**, A tumor sheet in a classical PTC (cPTC). **C**, A papilla in a cPTC. **D**, A pseudoinclusion in a cPTC.
as seen with either cPTC or infiltrative FVPTC, respectively (ie, tumors known to be associated with \(BRAF\) mutations), may not be easily discerned. Regardless of the explanation, their group of encapsulated FVPTCs had a relatively high rate of \(BRAF\) mutations; thus, it is not surprising that more cases had a preceding malignant diagnosis on FNA since \(BRAF\) mutations are usually associated with a malignant or SUS diagnosis on FNA.\(^{16,29,34,35}\) Finally, members of our group previously demonstrated that most malignancies in the AUS/FLUS category are FVPTCs.\(^{36}\) While these previous results may initially appear discordant with our current findings, they are actually not: although most malignancies in the AUS/FLUS category are FVPTCs, this does not require that the converse is true (ie, that most FVPTCs are preceded by an AUS/FLUS diagnosis).

Reclassifying NFVPTCs would decrease the positive predictive value of all FNA diagnoses. This is especially of concern in the SUS and malignant categories where the anticipated rate of malignancy is so high that most patients undergo total thyroidectomy. To gain insight into whether cases of NFVPTC can be distinguished from cPTC at the time of FNA, we evaluated a cohort of cytology specimens that had undergone molecular analysis for \(BRAF,\) \(RAS,\) \(PAX8/PPARG,\) and \(RET/PTC.\) NFVPTCs were significantly more frequently diagnosed as SUS on FNA and showed a predominantly microfollicular architecture, while cPTCs were significantly more frequently diagnosed as malignant and were associated with tumor sheets, papillae, and pseudoinclusions. No NFVPTCs had either papillae or pseudoinclusions present in the FNA specimen. Of the nine NFVPTCs with molecular analysis, three (33%) had a \(RAS\) mutation, one (11%) had a \(PAX8/PPARG\) rearrangement, and five (56%) lacked a \(BRAF,\) \(RAS,\) \(PAX8/PPARG,\) or \(RET/PTC\) alteration, whereas for the cPTCs with successful molecular analysis, 14 (61%) harbored a \(BRAF\) V600E mutation, one (4%) had an \(NRAS\) mutation, and 8 (35%) lacked a \(BRAF,\) \(RAS,\) \(PAX8/PPARG,\) or \(RET/PTC\) alteration. These results indicate that there are cytologic characteristics and molecular alterations that can be evaluated for on the preceding FNA that can aid in distinguishing NFVPTC and cPTC. This means that cytopathologists could potentially refine cytologic criteria to shift lesions with moderate nuclear features of PTC that lack papillae and pseudoinclusions into a lower risk diagnostic category (either AUS/FLUS or FOL). In addition, our results indicate that there could be an enhanced role for molecular testing of the SUS category to identify patients at greater risk of clinically significant disease. For example, identification of a \(BRAF\) V600E mutation could support the decision to proceed with a total thyroidectomy. In contrast, since \(RAS\) mutations in the setting of nuclear features of PTC are often associated with NFVPTCs, detection of a \(RAS\) mutation at the time of FNA diagnosis might indicate that a lobectomy is the best initial surgical approach, given that lobectomy alone has been proposed for patients with NFVPTCs diagnosed on the subsequent resection specimen.\(^{5}\)

In summary, we found that while many NFVPTCs are diagnosed as AUS/FLUS or FOL on FNA, essentially half are diagnosed as SUS. If terminology of these tumors changes and they are not classified as carcinoma, our findings indicate that this will have a significant impact not only on the rate of malignancy associated with AUS/FLUS and FOL categories but also the SUS and potentially the malignant category. Further larger studies across multiple institutions are indicated to validate our findings.

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