World J Surg (2012) 36:69–74
DOI 10.1007/s00268-011-1336-8

World Journal of Surgery

Incidence of Malignancy in Thyroid Nodules Determined to be Follicular Lesions of Undetermined Significance on Fine-Needle Aspiration

Gilberto V. Teixeira · Horacio Chikota · Thiago Teixeira · Gabriel Manfro · Sara I. Pai · Ralph P. Tufano

Published online: 5 November 2011

Abstract
Background Fine-needle aspiration (FNA) for thyroid nodules is the most important method for determining a diagnosis. The system for reporting results is based on a cytopathologic classification that stratifies the risk of malignancy.
Methods We retrospectively studied 197 patients who underwent FNA for diagnostic evaluation of a thyroid nodule and had their results reported as a follicular lesion of undetermined significance (FLUS) using the Bethesda classification system. The objective of the study was to analyze the incidence and histopathologic types of malignancy in these cases.
Results The final histopathologic breakdown is as follows: 65 cases (32.9%) of follicular adenoma, 81 cases (41.1%) of microfollicular adenomatoid nodule, 19 cases (9.6%) of microfollicular adenomatoid nodule on the background of thyroiditis, 17 cases (8.6%) of follicular carcinoma, 9 cases (4.6%) of follicular variant papillary carcinoma, and 6 cases (3.1%) of classic papillary carcinoma, for a 16.2% incidence of malignancy. Beyond these diagnoses in the FNA-biopsied nodules, we observed 29 cases (14.7%) of incidental ipsilateral papillary thyroid microcarcinoma (PTM) and 13 cases (6.6%) of incidental contralateral thyroid lobe PTM.
Conclusions This study observed a 16.2% incidence of thyroid cancer in the nodule designated FLUS compared to the 5 to 15% rate reported by the Bethesda FNA classification. The overall incidence of incidental PTM in the thyroid gland was 21.3%. These data support considering surgical intervention for at least diagnostic purposes in a patient with the FNAB diagnosis of FLUS.

Introduction

Fine-needle aspiration (FNA) for thyroid nodules is the most cost-effective method for determining a diagnosis that aids in treatment decision-making [1]. Patients who can benefit from FNA are those with thyroid nodules >1 cm and clinical (male sex, previous neck radiotherapy, family history of thyroid cancer), physical (palpable hard or fixed thyroid nodule, lateral neck mass), or sonographic findings suggestive of malignancy (microcalcification; hypoechoic, solid nodules; nodules with irregular or lobulated margins; intranodular vascularity; a taller-than-wide shape; signs of spread beyond the capsule; suspected lymph node alterations) [2].

This method provides a tailored strategy that helps clinicians and surgeons define the best treatment. The experience of cytologists and the associated use of ultrasonography (US) as guidance for FNA have improved the accuracy and safety of the method, and it is currently a popular method for initial diagnostic testing. However, its overall high sensitivity for

G. V. Teixeira (✉) · T. Teixeira
Department of Oncologic Surgery/Head and Neck Surgery CEPON, R. Presidente Coutinho, 579, Sala 304, Florianopolis, SC 88015-231, Brazil
e-mail: gteixei1@gmail.com

G. V. Teixeira · S. I. Pai · R. P. Tufano
Department of Otolaryngology/Head and Neck Surgery, Johns Hopkins Medical Institutions, 601 N Caroline Street, JHOC, 6th Floor, Baltimore, MD 21231, USA

H. Chikota
IMP Laboratory, R. Menino Deus, 63 Sala 14, Florianopolis, SC 88000, Brazil

G. Manfro
University Hospital UNOESC, R. Getulio Vargas, 2125, Joacaba, SC 89600, Brazil

suspicious lesions (94.1%) drops to 65.0% when analyzing only positive cases for cancer in the predictive test [3]. The recalculated sensitivity of the method is 86%, and the recalculated specificity found in the literature is 62% [4]. This gap could be explained by the use of different classification systems used to report the cytologic results, adding to the high interobserver variations in reporting results [5].

The development of a standard classification system with accepted terminology is a way to unify the diagnostic information obtained through FNA. The first step was done by the Bethesda cytopathologic six-category classification sponsored by the National Cancer Institute (NCI) in 2008 [6]. This classification is based on six suggested categories: benign, follicular lesion of undetermined significance (FLUS), follicular neoplasm, suspicious for malignancy, malignant, nondiagnostic [6].

One of the most controversial points is to define the best management for patients in which cytology shows an indeterminate or suspicious (for neoplasm and malignancy) pattern on FNA. Many reports in the literature suggest that the cytologic description alone may not be enough to define the right treatment strategy. Many ancillary studies were supplemented with the FNA trying to improve the accuracy. In these cases, the radiologic findings, cytologic features, and expression of cell markers are proposed to work as a diagnostic adjunctive tool in the evaluation of those nodules but with limited relevance [7–9].

The Bethesda system has implied risks of malignancy that influence management paradigms based on previous studies done before the NCI FNA Conference in 2008 [6]. As noted, the Bethesda system favored a six-category model introducing an optional group defined as FLUS, or "atypia of undetermined significance" (AUS). Some of the first publications addressing this category suggested a risk of malignancy ranging from 5 to 10% [6]. Recent publications have reported an increase in the risk incidence of malignant disease ranging from 5 to 15% in this category [10, 11]. Based on this stratified system, the decision-making for "suspicious for malignancy" and "follicular neoplasm" groups seems easy. These two categories represent a risk of malignancy ranging from 50 to 75% and 20 to 30%, respectively. In these situations the indication for a more aggressive treatment based on a surgical approach is more reasonable [10].

For the FLUS category, the correlation with the patient's history, sonographic findings, and patient's expectations leads to a more personalized decision-making process [5]. It is recommended that patients in the FLUS category undergo a repeat FNA, which may decrease the risk of unnecessary surgery [12]. Interestingly, when patients with a FLUS FNA diagnosis are submitted to surgical excision of the thyroid gland—based on a repeat FNA showing FLUS or with worrisome clinical or radiologic findings—they experience an overestimated incidence of cancer, from 20 to 25% [13]. Despite being an optional category, with a suggested utilization in an institution's reported FNA results of $< 7\%$ [6], the elimination of this category from the reporting system would decrease the sensitivity of the thyroid FNA and would increase both false-positive and false-negative rates [14]. The real incidence of malignant disease in FLUS cases is biased by the low rate of patients submitted to a surgical treatment. For these patients showing FLUS in their FNA results, the average incidence of malignant disease ranges between 4.95 and 35.0% [14–17].

Material and methods

We retrospectively evaluated 307 patients who had undergone total thyroidectomy between January 2005 and June 2009 and identified a subset of patients with cytologic findings from their preoperative FNA that demonstrated a follicular-patterned thyroid lesion that were intermediate between benign and neoplastic. These cases were re-reviewed and reclassified, with 197 patients diagnosed with FLUS according to the Bethesda system classification for thyroid FNA (2008) by the same pathologist (H.C.).

Entry criteria for this study were patients who had undergone total thyroidectomy, had at least one FNA showing FLUS based on the Bethesda system classification for thyroid FNA (2008), and had no history of previous neck irradiation. Patients who had had one or repeated FNAs showing FLUS as the highest cytologic criteria for indication of surgery were analyzed in this group. The indications for surgery were based on FNA criteria, and the indications for total thyroidectomy were based on suspicious sonographic findings, the presence of multiple nodules, the presence of clinical hypothyroidism, and the patient's preference. Patients submitted to thyroid lobectomy were not included in the study. Patients were sent for surgical treatment at the Dom Joaquim Clinic, Florianopolis, Brazil.

The US-guided FNA, analysis of the cytologic smears, classification, and final histopathologic report were done by the same pathologist (H.C.). FNA of all thyroid nodules was performed under US guidance, with the patient receiving local anesthesia. One to three aspirates of the suspected nodule were acquired using 22-gauge needles on a 10-ml syringe with pistol grip-like holders. One to three slides per biopsy were prepared with ethanol fixation, and the remaining material in the needle or syringe was preserved by ethanol fixation for paraffin processing and histologic (cell block) examination. At the time of the biopsy, one slide was stained with methylene blue to ascertain the quality of the material under microscopic evaluation. In the laboratory, the slides were stained via the Papanicolau

procedure, and the paraffin cell blocks were stained with hematoxylin-eosin (H&E). FLUS was diagnosed when the aspirates contained a predominance of microfollicles with follicular or Hurthle cells, and cellular dissociation on a background with scant or no colloid; there were occasional follicular cells with enlarged, pale, grooved nuclei in an otherwise benign aspirate; or the cytologic findings were not convincingly benign nor sufficiently suspect for a designation of any other type of follicular category (suspicious for follicular neoplasm).

Demographic data of the patients including sex, age at surgical treatment, histopathologic features, final histopathologic report, focality, and the presence of occult disease were studied. All surgical specimens were subjected to histopathologic examination with tissue sections every 1.5 mm.

The effects of sex and age were evaluated by univariate analysis using the Pearson χ^2 and Fischer's exact test. A P-value < 0.05 was considered statistically significant. The results of the tumor size, histopathologic study, and focality were described. All statistical analyses were performed by SPSS version 19.0 for Windows software (SPSS, Chicago, IL, USA).

Results

Of the 197 patients, 15 were men and 182 were women. The sex distribution showed a prevalence of disease in women. No association with an increased risk of malignancy was observed (Pearson's χ^2: $P = 0.637$; Fisher's exact test: $P = 0.710$) (Table 1). The evaluation of age, divided in groups of those ≥50 years and those <50 years showed 98 (49.7%) patients in the first group and 99 (50.3%) in the second. The age stratification was not associated with an increased risk of malignancy (Pearson's χ^2: $P + 0.313$; Fischer's exact test: $P = 0.334$) (Table 1). The sizes of the nodules that were submitted to FNA were grouped according to the T clinical classification of the American Joint Committee on Cancer (AJCC) staging system (2002). Most were located in the group <2.0 cm (71.6%). A comparison between these groups and the risk of malignancy in the FNA nodules did not express an association (Pearson's χ^2: $P = 0.468$) (Table 1).

The final histopathologic breakdown included 81 cases (41.1%) of microfollicular adenomatoid nodule, 65 cases (32.9%) of follicular adenoma, 19 cases (9.6%) of microfollicular adenomatoid nodule on the background of thyroiditis, 17 cases (8.6%) of follicular carcinoma, 9 cases (4.6%) of follicular variant papillary carcinoma, and 6 cases (3.1%) of classic papillary carcinoma, for a 16.2% incidence of malignancy (Table 2). In addition to these diagnoses in the FNA-biopsied nodules, we observed 29 cases (14.7%) of incidental ipsilateral papillary thyroid microcarcinoma (PTM) and 13 cases (6.6%) of incidental contralateral thyroid lobe PTM (Table 3).

The 1.5-mm sections of the entire thyroid gland demonstrated an association with multifocal malignant disease in 9 of 15 cases of PTC and 2 of 17 cases of follicular carcinoma, with incidences of multifocal disease of 60.0 and 11.7%, respectively. In 31 cases (15.7%) where the FNAed lesion was read as benign, on final surgical pathology an incidental (occult) PTM was detected elsewhere in the thyroid gland.

Table 1 Demographic and macroscopic features in 197 cases of FNA with FLUS and relation with the risk of malignant disease in the final anatomicopathologic report

Variable	Malignant nodule (no.)	Nonmalignant nodule (no.)	P*	P**
Sex			0.637	0.710
Male	3 (20%)	12 (80%)		
Female	29 (15%)	153 (85%)		
Age (years)			0.313	0.334
<50	18 (18%)	80 (82%)		
≥50	13 (13%)	86 (87%)		
Size (cm)			0.468	_
≤2	19 (13%)	122 (87%)		
>2.0–4.0	9 (21%)	33 (79%)		
>4.0	3 (21%)	11(79%)		

* Pearson's χ^2

** Fisher's exact test

FNA fine-needle aspiration; *FLUS* follicular lesion of undetermined significance

Discussion

The FNA was popularized in Europe and North America during the 1990s as a method that could facilitate a tailored strategy to define the treatment of patients with thyroid nodules. The aim of FNA is to stratify the risk for malignancy in the targeted nodule. Despite its high sensitivity for detecting malignant disease (89–100%), the specificity

Table 2 Final histopathologic report in 197 FNA nodules

Histopathology	No.	%
Microfollicular adenomatoid nodule	81	41.1
Follicular adenoma	65	33.0
Microfollicular adenomatoid nodule in thyroiditis	19	9.6
Follicular carcinoma	17	8.6
Classic papillary carcinoma	6	3.1
Follicular variant papillary carcinoma	9	4.6

Table 3 Distribution of 74 malignant tumors and relation to FNA nodule

Relation to FNA nodule	No.	% (Based on 197 cases)
Same FNA nodule	32	16.2
Incidental non-FNA ipsilateral PTM	29	14.7
Incidental non-FNA contralateral PTM	13	6.6

PTM papillary thyroid microcarcinoma

varies from 69 to 100% [18]. This difference is based on the presence of an intermediate group of diagnoses [3].

The FNA category of "follicular pattern neoplasms" defines a group of cytologic findings that supports the histopathologic diagnosis of hyperplastic nodules, follicular adenoma, follicular carcinoma, and follicular variant of PTC [19]. These cytologic findings are also reported in thyroid nodules harboring nodular goiter, classic PTC, Hashimoto's thyroiditis, Hurthle cell adenoma, Hurthle cell carcinoma, metastatic neuroendocrine carcinoma, and others [2, 19].

The final diagnosis of malignancy of FNA-defined follicular lesions is related to the histopathologic finding of vascular and capsular invasion, leading most practitioners to perform surgery for a definitive diagnosis [12] Various terms and classification schemes are employed to define this category of FNA. Follicular lesion, atypical follicular lesion, and follicular neoplasm are the most commonly used terms [12]. Most professional societies and associations provide classifications for reporting FNA results that are based on five or six groups of results [20]. The inclusion of two subgroups to report follicular patterns in FNA cytologic results seems to provide a more accurate result.

The definition of the FLUS subgroup by the Bethesda system for reporting FNA results states that it "is a heterogeneous category that includes cases in which the cytologic findings are not convincingly benign, yet the degree of cellular or architectural atypia is not sufficient for an interpretation of follicular neoplasm" [6]. This category was introduced as optional and, when utilized, would ideally represent <7% of all thyroid FNA interpretations at an institution [6]. Assuming that 6 to 55% (mean 24%) of the FNA results are related to a follicular (indeterminate) pattern [4], 7% of the total results could represent almost one-fourth of the results in this group.

The use of FLUS to report FNA results in the literature ranges from 2.1 [14] to 12.0% [21] of the total number of reports. Jo et al. [10] studying 3,080 FNA results, reported a frequency of 3.4% for FLUS and 9.7% for the follicular neoplasm category, showing a rate of 1:3, respectively, among this follicular (indeterminate) lesion group. This group is a well-known gray zone in cytology [14] and should be associated with an unexpected overuse in the literature based on the fact that many laboratories are using this category of results on a large scale. The criteria for using the term "follicular lesion of undetermined significance" is related to an increased interobserver and intraobserver variability [4, 14, 22]. Atypical cells diagnosed in FNA smears should reflect the presence of an elevated potential risk for malignancy in the sample. Cytologic features such as nuclear atypia increase the risk of suspicion for malignancy when observed in an FNA sample, with a variable predictive value. Sahin et al. analyzed 86 patients with cytologically indeterminate thyroid nodules who underwent thyroidectomy and observed a 51.7% prevalence of malignancy in patients with atypical cell cytology and a 15.0% prevalence of malignancy in patients with follicular neoplasm cytology [23]. In that study, the follicular lesion with atypia was assigned to cellular aspirates with scant or absent colloid with cytologic atypia including pleomorphism, enlarged nuclei, nuclear grooves, coarse or irregular chromatin, prominent nucleoli, or atypical or numerous mitotic figures. In this atypical cell group, they found an incidence of PTC corresponding to 83.3%. The challenge when reporting FNA results with atypical cells is to distinguish the group that expresses atypical cells of undetermined significance from the group with atypical cells that may be related to an increased risk of malignancy, mainly PTC. Numerous conditions (e.g., thyroiditis, posttreatment effects, adenomatoid nodules) can be accompanied by marked cellular atypia. Also, some cases are placed in the FLUS category based on a compromised smear expressing low cellularity, poor fixation, and obscuring blood [6].

Based on this scenario, the proposal for a repeat FNA after 3 to 6 months seems reasonable to clarify the best approach for these patients. Baloch et al. [24] studying the role of repeat FNA for an "indeterminate for neoplasm" group, observed a 48% incidence of malignancy among the patients submitted to the repeat biopsy and who had a persistently indeterminate or suspicious/positive result. In our study, the many patients submitted to surgery after repeat FNA findings showed persistent FLUS.

The suggested rate for malignancy in this category ranges from 5 to 15% [10, 11]. The number of patients whose FNAs are reported as FLUS and undergo surgical treatment is between 22.9 and 58.3% [14–16]. For these patients, the average incidence of malignant disease ranges from 4.95 to 35.0% [14–17]. In our study, the overall incidence of malignant disease in the FNA-biopsied nodules read as FLUS was 16.2%, a higher value than the suggested 5 to 15%. This higher incidence of malignancy among our FLUS cases was biased by the fact that we studied only patients who progressed to surgery and by excluding those who were followed clinically. The suggested incidence of malignancy for all patients having FLUS in their FNA results ranges from 5 to 15% [10, 11]; but for those submitted to surgical treatment this incidence can reach 25% [13]. Some clinical factors (e.g., sex, age, size of

the nodule) should contribute to delineating a risk group of patients who are carriers of an FNA-indeterminate group and have an elevated incidence of malignancy [25]. We found no statistically significant association between the size of the nodule, age, or sex of the patient with elevated risk of malignancy among those designated FLUS.

The incidence of incidental PTM associated with FLUS nodules is not specifically defined in the literature and not often validated [10]. The high prevalence of incidental PTM in autopsy cases and that is related to other thyroid pathology is not a new finding. In autopsy cases the incidence ranges from 0.01 to 35.6%; and in recent reports of thyroidectomy performed for thyroid disease other than diagnosed malignancy, it ranges from 3.1 to 21.0% [26]. The impact of this association with other thyroid disease needs to be clarified. In our study, the incidence of incidental PTM was 21.3%. It was also related to the thin 1.5 mm thickness of the anatomopathologic slices of the thyroid gland. We were able to analyze the association between incidental PTM and FLUS nodules only in patients submitted to total thyroidectomy. Therefore, we did not include patient who underwent thyroid lobectomy only. The incidence of contralateral PTM (opposite to the lobe harboring the FLUS nodule) was 6.6%. This is not enough to justify total thyroidectomy for all patients with nodules that have a FLUS cytopathology report. The indication for total thyroidectomy in a patient with a FLUS diagnosis should be based on clinical and radiologic examination findings along with the patient's preference.

Conclusions

The FLUS group in the Bethesda system for reporting FNA results of thyroid nodules constitutes a controversial category that needs more clarification to be used as a reproducible and well accepted risk stratification parameter for thyroid cancer. The 16.2% incidence of malignancy in FNA nodules read as FLUS in patients who underwent surgery and the 21.3% rate of associated incidental PTM demonstrate the importance of offering surgical treatment in selected cases.

Acknowledgments The authors thank Barbara Silver, BS, MBA for her contribution to this study.

Conflicts of interest The authors report no potential conflicts of interest in this study.

References

1. Cooper DS, Doherty GM, Haugen BR et al (2009) Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19:1167–1214
2. Layfield LJ, Cibas ES, Gharib H et al (2009) Thyroid aspiration cytology: current status. CA Cancer J Clin 59:99–110
3. Seningen JL, Nassar A, Henry MR (2010) Correlation of thyroid nodule fine-needle aspiration cytology with corresponding histology at Mayo Clinic, 2001–2007: an institutional experience of 1,945 cases. Diagn Cytopathol Dec 3. [Epub ahead of print]
4. Lewis CM, Chang KP, Pitman M et al (2009) Thyroid fine-needle aspiration biopsy: variability in reporting. Thyroid 19:717–723
5. Baloch ZW, LiVolsi VA, Asa SL et al (2008) Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. Diagn Cytopathol 36:425–437
6. Baloch ZW, Cibas ES, Clark DP et al (2008) The National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference: a summation. Cytojournal 5:6
7. Maruta J, Hashimoto H, Suehisa Y et al (2010) Improving the diagnostic accuracy of thyroid follicular neoplasms: cytological features in fine-needle aspiration cytology. Diagn Cytopathol 39:28–34
8. Sapio MR, Posca D, Raggioli A et al (2007) Detection of RET/PTC, TRK and BRAF mutations in preoperative diagnosis of thyroid nodules with indeterminate cytological findings. Clin Endocrinol (Oxf) 66:678–683
9. Yoon JH, Kwak JY, Kim EK et al (2010) How to approach thyroid nodules with indeterminate cytology. Ann Surg Oncol 17:2147–2155
10. Jo VY, Stelow EB, Dustin SM et al (2010) Malignancy risk for fine-needle aspiration of thyroid lesions according to the Bethesda system for reporting thyroid cytopathology. Am J Clin Pathol 134:450–456
11. Layfield LJ, Cibas ES, Baloch Z (2010) Thyroid fine needle aspiration cytology: a review of the National Cancer Institute state of the science symposium. Cytopathology 21:75–85
12. Faquin WC, Baloch ZW (2010) Fine-needle aspiration of follicular patterned lesions of the thyroid: diagnosis, management, and follow-up according to National Cancer Institute (NCI) recommendations. Diagn Cytopathol 38:731–739
13. Cibas ES, Ali SZ (2009) The Bethesda system for reporting thyroid cytopathology. Am J Clin Pathol 132:658–665
14. Shi Y, Ding X, Klein M et al (2009) Thyroid fine-needle aspiration with atypia of undetermined significance: a necessary or optional category? Cancer 117:298–304
15. Yang J, Schnadig V, Logrono R et al (2007) Fine-needle aspiration of thyroid nodules: a study of 4,703 patients with histologic and clinical correlations. Cancer 111:306–315
16. Yassa L, Cibas ES, Benson CB et al (2007) Long-term assessment of a multidisciplinary approach to thyroid nodule diagnostic evaluation. Cancer 111:508–516
17. Deandrea M, Ragazzoni F, Motta M et al (2010) Diagnostic value of a cytomorphological subclassification of follicular patterned thyroid lesions: a study of 927 consecutive cases with histological correlation. Thyroid 20:1077–1083
18. Castro MR, Gharib H (2005) Continuing controversies in the management of thyroid nodules. Ann Intern Med 142:926–931
19. Greaves TS, Olvera M, Florentine BD et al (2000) Follicular lesions of thyroid: a 5-year fine-needle aspiration experience. Cancer 90:335–341
20. Crippa S, Mazzucchelli L, Cibas ES et al (2010) The Bethesda system for reporting thyroid fine-needle aspiration specimens. Am J Clin Pathol 134:343–344 author reply 345
21. Rabaglia JL, Kabbani W, Wallace L et al (2010) Effect of the Bethesda system for reporting thyroid cytopathology on thyroidectomy rates and malignancy risk in cytologically indeterminate lesions. Surgery 148:1267–1272 discussion 1272–1273

22. Faquin WC (2009) Diagnosis and reporting of follicular-patterned thyroid lesions by fine needle aspiration. Head Neck Pathol 3:82–85
23. Sahin M, Gursoy A, Tutuncu NB, Guvener DN (2006) Prevalence and prediction of malignancy in cytologically indeterminate thyroid nodules. Clin Endocrinol (Oxf) 65:514–518
24. Baloch Z, LiVolsi VA, Jain P et al (2003) Role of repeat fine-needle aspiration biopsy (FNAB) in the management of thyroid nodules. Diagn Cytopathol 29:203–206
25. Raparia K, Min SK, Mody DR et al (2009) Clinical outcomes for "suspicious" category in thyroid fine-needle aspiration biopsy: patient's sex and nodule size are possible predictors of malignancy. Arch Pathol Lab Med 133:787–790
26. Roti E, degli Uberti EC, Bondanelli M et al (2008) Thyroid papillary microcarcinoma: a descriptive and meta-analysis study. Eur J Endocrinol 159:659–673