Published in final edited form as:

Ann Surg Oncol. 2013 January ; 20(1): . doi:10.1245/s10434-012-2601-2.

The Management of Thyroid Nodules with Atypical Cytology on Fine Needle Aspiration Biopsy

Sushruta S. Nagarkatti, MD¹, William C. Faquin, MD, PhD², Carrie C. Lubitz, MD¹, Dieter Morales Garcia¹, Giuseppe Barbesino, MD³, Douglas S. Ross, MD³, Richard A. Hodin, MD¹, Gilbert H. Daniels, MD³, and Sareh Parangi, MD¹

¹Thyroid Cancer Laboratory, Endocrine Surgery Unit, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA

²Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

³Thyroid Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Abstract

Background—Fine needle aspiration biopsies (FNAB) of the thyroid categorized as atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) is a newly defined category per the recent Bethesda guidelines. In this study, we sought to assess the characteristics and treatment of patients with an AUS/FLUS FNAB at our institution. Additionally, we evaluated the utility of the recommended three-month timing of repeat FNAB.

Methods—A retrospective study of all patients with an FNAB categorized as AUS/FLUS between 1/2005–12/2007 at an academic, tertiary care center. Clinical, cytological and ultrasound variables were compared among management groups. Differences in patients receiving repeat FNAB before or after a three-month interval were also compared.

Results—203 patients of the 5,391 FNAB's performed at our institution met the Bethesda criteria for AUS/FLUS. 62% were triaged directly to surgery (group I), 25% had a repeat FNAB (group II) and 13% were observed (group III). Younger ($p = 0.006$), male patients ($p = 0.04$) were more likely to go directly to surgery. 2.4% of males were observed vs. 16% of the women ($p = 0.04$); Timing of repeat FNAB (< or ≥ 3 months) did not alter the results of the second FNAB ($p = 0.73$). The overall rate of malignancy in patients undergoing surgery was 15.7%.

Conclusion(s)—Current practice relies mainly on established prognostic factors such as gender, and age. Timing of repeat biopsy did not alter management, repeat FNAB diagnosis, or rate of malignancy in our cohort.

Keywords

Atypia of Undetermined Significance; Thyroid Nodules with Atypia; Fine Needle Aspiration Biopsy

Introduction

Thyroid nodules are common and thyroid cancers are relatively rare. FNAB is the gold standard for the evaluation of thyroid nodules, and plays a key role in management.^[1] Classification and reporting systems for thyroid FNAB results are not standardized across, and often within, institutions^{[2] [3]}. A revised classification system for reporting thyroid FNAB cytology was proposed at the National Cancer Institute(NCI) State of Science conference (NCISSC) in 2007^[2]. This led to the Bethesda System for Reporting Thyroid Cytopathology (BS), which provides a revised six tiered reporting system with an emphasis on the risk of malignancy associated with each category (Table 1) ^[4]. The category “indeterminate for malignancy” was subdivided into “suspicious for malignancy”, “follicular neoplasm” and “atypia of undetermined significance/follicular lesion of undetermined significance” (AUS/FLUS) based on morphology, cytology and corresponding risk of malignancy, allowing further stratification of lesions with a follicular pattern, with or without nuclear atypia.

The category AUS/FLUS is heterogeneous: specimens placed in this category cannot be characterized as benign due to the presence of follicular cells with nuclear or architectural atypia, yet the atypia is insufficient for categorization as suspicious for malignancy, malignant or follicular neoplasm. The risk of malignancy for an AUS/FLUS FNAB continues to be studied; however, the estimated risk is reported as 5% to 19 % of those referred for surgery^[4–7]. The overall risk of malignancy will be higher when the denominator is based on patients referred for surgery and lower when the denominator consists of all diagnosed patients ^[8]. As per the synopsis of the NCISSC , patients with an AUS/FLUS FNAB should undergo a second FNAB after three months based on clinical correlation and/or ultrasound findings^[4, 7, 9, 10]. Performing a repeat FNAB before three months is thought to increase the chance of reparative atypia of follicular cells with the potential for false positive diagnosis of malignancy^[2]; However, there is little objective data to support this recommendation.

While for the Bethesda System thyroid FNAB cytopathology classifications is new, a similar system has been in use at our institution for the last 15 years. We hoped to use the data from our experience with this category to guide others new to using this scheme. The aim of our study was to determine how patients AUS/FLUS results were treated at our institution and whether they were in fact always having a repeat FNAB after a three month waiting period. Our hypothesis was that waiting 3 months to repeat the FNAB in patients with an initial diagnosis of AUS/FLUS is not necessary. At our institution there has been no mandated waiting period prior to repeat FNAs. Clinicians have used their own criteria or judgment as to the timing of the repeat FNA. We reviewed the management of patients at our institution with AUS/FLUS FNABs. We determined if various clinical, morphological and ultrasound characteristics, or timing of the repeat biopsy played a role in directing whether the next step in this new category of patients should be.

Methods

Patient cohort

Patient accrual/Inclusion criteria: An IRB-approved search of the Massachusetts General Hospital cytology electronic database was performed for 1/2005–12/2007; a total of 5,391 thyroid FNABs were performed; 254 patients had their FNAB interpreted as AUS/FLUS (4.7%), as defined by the BS proposed by the NCI^[2, 4, 10] and included FNAB’s interpreted as “atypical”, “atypia of undetermined significance”, “follicular lesion of undetermined significance”, and “rule-out follicular neoplasm”. For each patient additional fine print notes

provided in the text of the report were also reviewed by our one cytopathologist (WCF). 51 patients were excluded due to the lack of adequate follow-up.

Of the 203 remaining patients in our cohort, 162 were female (80%) and 41 were male (20%) with a mean age of 53 years (SD $\pm$ 13). We divided our cohort into three distinct groups based on the next management step after initial AUS/FLUS FNAB (Figure 1).

Group I: Patients referred directly for surgery without a repeat FNAB

Group II: Patients with a repeat FNAB at any time following initial FNAB

- Subgroup IIa: FNAB performed < 3 months after initial FNAB
- Subgroup IIb: FNAB performed $\geq$ 3 months after initial FNAB

Group III: Patients that received only observation following the initial AUS/FLUS FNAB. In this category adequate follow-up was defined as at least one 6 month follow up endocrinology visit with ultrasound.

Timing of repeat FNA (< 3 months or $\geq$ 3 months after initial FNAB) was analyzed in Group II. Each group/subgroup was compared based on:

1. *Demographic and clinical characteristics*: age, gender, history of radiation, presence or absence of Hashimoto's thyroiditis, previous thyroid surgery, TSH levels, and family history of thyroid disease
2. *Ultrasound characteristics*: echogenicity, margins, size, microcalcifications, and central flow.
3. *Final pathology*: On final pathology only the index nodule was considered as benign or malignant. Patients with papillary microcarcinomas were not considered to have malignant thyroid disease unless the index nodule with AUS/FLUS was malignant.

The FNABs were performed under ultrasound guidance using 25–27 gauge needles to make three to four passes. Six-eight alcohol-fixed smears underwent Papanicolaou staining while the remainder was prepared for ThinPrep by rinsing the needle in Cytolyte^[7]. All cytology reports and slides were re-reviewed by one cytopathologist (WCF) and were found to be concordant with the original reading.

Statistical analysis

A univariate analysis using a Pearson Chi-square test for discrete variables and ANOVA for continuous variables was performed to determine significance.

Results

Patients referred for repeat FNAB or directly to surgery have similar rates of malignancy on final pathology

Of 203 patients in our cohort with AUS/FLUS on initial FNAB, 125 patients (62%) were in Group I (referred directly to surgery), 51 patients in Group II (repeat FNAB) (25%), and 27 patients (13%) were in Group III (observed). Table 2 reveals the data analysis from these groups. When the three groups were compared for demographic and clinical characteristics, patients in Group I were significantly younger and more likely to be male compared to the other groups. All other clinical and US imaging characteristics (history of radiation, presence or absence of Hashimoto's thyroiditis, previous thyroid surgery, TSH levels, and family history of thyroid disease, echogenicity, nodule margins, nodule size, presence of microcalcifications, and central flow) were not statistically different between all groups.

Group I—All patients in group I (n=125) were referred directly to surgery after the initial FNAB showed AUS/FLUS. 105 (84%) had a benign diagnosis on final pathology. Twenty patients (16%) had a malignancy, classical papillary thyroid cancer was seen in ten patients, follicular variant of papillary cancer(FVPTC) in four, five had minimally invasive follicular carcinomas and one patient had a Hürthle cell carcinoma.

Group II—All 51 patients in this group had a repeat FNAB. The repeat FNAB was benign in 22 patients and again read as AUS/FLUS in 17 patients, 6 patients had non-diagnostic specimens, and 3 patients each were diagnosed as follicular neoplasm and suspicious for malignancy. Surgery following a repeat biopsy was done in 26/51 patients (51%) while 18 patients (35%) were then observed. Seven patients (14%) were lost to follow up after the repeat biopsy. In patients subjected to a repeat FNAB prior to surgical intervention, final pathology showed malignancy in four patients (15%) while the remaining 22 (85%) had benign thyroid disease. As listed in Table 3, of these four malignant tumors, two were FVPTC, and two were minimally invasive follicular carcinomas. The rate of malignancy on final pathology was not statistically different in this group when compared to those directed to surgery without repeat FNAB.

Group III—All 27 patients in this category were observed and were not subjected to surgery or repeat FNAB within at least a 6 month follow up.

A three month waiting period prior to any repeat FNAB does not improve the diagnostic yield or alter patient care algorithm

Of those patients who had repeat FNAB in group II, 30 patients had their repeat FNAB within three months of the initial biopsy (early repeat FNAB < 3 months) while 21 patients had a late repeat FNAB (≥ 3 months). The median number of days between initial and repeat FNAB for patients that had an early repeat FNAB was 27 days. All five patients with a history of radiation exposure or therapeutic radiation had an early repeat FNAB. In these two subgroups, demographic and ultrasound data showed no statistically significant difference except in those with a history of therapeutic radiation exposure who were more likely to undergo repeat biopsy within three months of initial biopsy ($p < 0.05$). None of the other factors --age, gender, and morphological pattern on the initial AUS/FLUS, FNAB diagnosis of a second nodule, Hashimoto's disease, nor the ultrasound characteristics of the nodule, such as size, central flow, microcalcifications, and cystic nature-- were significant in determining whether a repeat biopsy was done before or after three months.

The timing of the repeat biopsy did not significantly alter the distribution of biopsies ($p = 0.73$). As shown in table 4, patients who were biopsied within 3 months of the initial biopsy did not have an increased rate of non-diagnostic or AUS/FLUS in their second biopsy compared to those who had repeat biopsies after 3 months. In fact, none of the repeat FNAB's had an FNAB diagnosis of malignancy. Nineteen patients (19/30) undergoing FNAB prior to 3 months underwent surgery; the final pathology was malignant in 2 and benign in 17 (90%). In the late repeat FNA subgroup, seven patients (7/21) were referred for surgery following a repeat biopsy and this was not statistically different from those patients who had their repeat biopsy done prior to 3 months. In all patients undergoing surgery after late repeat FNAB, final pathology was malignant in only one patient (14%), and benign in 6 (86%) patients.

Discussion

In general, the recommendation for management of patients with AUS/FLUS is to perform a repeat FNAB three or more months after the initial FNAB [2, 10]. In our retrospective study,

we analyzed our institutional experience in management of patients with FNAB's reported as AUS/FLUS; many of these patients were evaluated prior to the publication of the NCISSC in 2007⁽³⁾ since our institution has used a similarly tiered system for 15 years. We evaluated several possible variables that may have influenced the subsequent management of these patients and determined whether all patients had a repeat FNAB as recommended. We also evaluated whether it was necessary to wait three months prior to repeating the FNAB.

While the BS advocates the creation of a category termed AUS/FLUS, it also recommends that this category be used sparingly, noting prevalence rates of less than seven percent; higher rates may represent overuse of this category. Most series report that three to six percent of FNAB's performed fall into the AUS/FLUS category^[4, 11, 12]. However Layfield et al (2010) reviewed data from 3 University Hospitals and found that the prevalence of AUS/FLUS varied from 2.5 to 28.6 % among pathologists and from 3.3 to 14.9 % among institutions. In our series we found the prevalence of AUS/FLUS to be around five percent, which is well within the recommendations. In our cohort more than half of the patients were referred directly for surgery without a repeat FNAB; these were more likely to be younger and male than patients who had a repeat FNAB. The Bethesda recommendations also note that certain patients may be sent directly for surgery or be observed without a repeat FNAB based on certain clinical and ultrasound features^[4]. However, in our study, ultrasound features such as micro- calcifications, irregular margins, and marked hypoechogenicity^[13, 14] did not appear to influence the decision to repeat the FNAB, observe the patient or refer the patient for surgery. Additional variables such as a family history of thyroid disease, the presence of Hashimoto's thyroiditis, serum TSH concentration or a history of head or neck irradiation also did not appear to influence this decision. The choice of surgery versus repeat FNAB or observation was primarily based on the patient's age, the patient's gender, the clinical judgment of the endocrinologist and/or surgeon as well as patient preference. The cytoarchitecture of the specimen also appears to have played a role in referring patients directly for surgery. When the words "microfollicles" was written in the fine print of the cytology report, more patients were referred to surgery than when compared to use of the words "macrofollicles". Aspirates with microfollicles in a background of scant colloid and sparse or low cellularity were also more likely to have surgery without a second FNAB. These two patterns of cytoarchitecture are both consistent with the description of specimens placed in the AUS/FLUS category^[4]. The BS describes specimens with adequate cellularity and a mixed pattern with a relative predominance of microfollicles compared to macrofollicles as AUS/FLUS. This is also true for specimens with low cellularity and scant colloid that have a relative predominance of microfollicles. In both of these scenarios, the cytoarchitectural pattern with a slight increase of microfollicles is insufficient to interpret as suspicious for a follicular neoplasm^[4], but based on our data, may be worrisome enough for the clinician to refer patients directly for surgery. The BS also suggests that certain patients may be sent directly for surgery or be observed without a repeat biopsy based on certain clinical and ultrasound features^[4]. However, in our study, ultrasound features such as micro- calcifications, irregular margins, and marked hypoechogenicity^[13, 14] did not appear to play a role in the next step in management, deciding between surgery vs. a repeat biopsy or observation alone. A family history of thyroid disease, Hashimoto thyroiditis, TSH levels and a history of radiation exposure also did not play a role in the decision process following the diagnosis of AUS/FLUS at our institution.

The Bethesda classification is designed to provide a uniform reporting system for FNAB, with an implied risk of malignancy associated with each diagnostic category. The reported risk of malignancy for AUS/FLUS ranges from 5 – 19 % of patients who have surgery. However, there are serious limitations to this risk assessment. The percentage of AUS/FLUS

patients who undergo surgery is often lower than other more suspicious FNAB categories. Usually, surgery is only performed in patients deemed to be at a high risk for malignancy or those that have had a second AUS/FLUS biopsy. The risk of malignancy in this category therefore varies depending upon whether the denominator is the population undergoing surgery or the entire initial AUS/FLUS group^[4, 8]. In our study, almost 75% of the patients of our 203 patients had surgery, either with or without a repeat FNAB. The overall malignancy rate in our cohort was 15.7 %. This rate of malignancy is consistent with the risk of malignancy reported for AUS/FLUS in other studies^[4]. In our series, the risk of malignancy was similar in those patients referred directly for surgery (16%) and those who had surgery following a repeat FNAB (15%). Therefore, referring patients for surgery based on clinical characteristics such as age and gender may help identify patients who need surgery almost as well as a repeat biopsy. It may be useful to consider surgery without a repeat FNAB for younger, male patients.

In our series half of the AUS/FLUS patients who went directly to surgery had conventional papillary thyroid carcinoma. The remainder had follicular variant of papillary thyroid carcinomas (FVPTC) (20%) or follicular (FTC) or oxyphilic follicular thyroid carcinomas (30%). Of the four malignancies found after repeat FNAB, 2 were FVPTC and two were FTC. The repeat FNAB was AUS/FLUS in three of these patients. Three out of four of these patients had repeat FNAB diagnosed as AUS/FLUS. When the initial diagnosis on FNAB is AUS/FLUS a repeat FNA may help clarify the situation. For example Yassa et al reported that a repeat FNAB yielded a benign diagnosis in 50% of patients, a more suspicious diagnosis in 32 % of patients and AUS/FLUS in around 20 %. ^[11]. In our study we found that approximately 33% of patients had an AUS/FLUS diagnosis on repeat biopsy, with almost all of these patients being referred for surgery.

It is recommended that one wait three or more months before repeating an FNAB after an initial FNAB categorized as AUS/FLUS ^[2, 10]. This waiting period is designed to avoid any atypical reparative changes that may interfere with the interpretation of the repeat biopsy and lead to an increased diagnosis of malignancy or suspicious for malignancy. ^[2]. However, there are few data to support this recommendation. We found no statistically significant difference in outcome when an FNAB was performed less than or more than 3 months after the initial AUS/FLUS FNAB. Specifically we did not find an increase in the number of biopsies that were suggestive of a malignant diagnosis in either subgroup. We also found that the proportion of specimens diagnosed as AUS/FLUS on a repeat biopsy was not significantly increased in patients who had a biopsy at less than three months. Therefore, we interpret these retrospective data as evidence that the timing of a repeat FNAB (i.e. 3 months versus > 3 months) may not be of clinical importance. The timing of the repeat FNAB did not correlate with usual worrisome clinical or ultrasound characteristics but was more likely to be performed in less than 3 months for patients with a history of prior head or neck irradiation.

Our study has several limitations. This was a retrospective study with 51 patients were excluded due to inadequate follow-up. We were unable to account for the variable of patient preference, a major determinant in clinical practice which might bias management decisions. The AUS/FLUS category is by its nature heterogeneous. Accurate division of specimens into groups based on cytomorphologic architecture or degree of atypia is difficult since the exact number of microfollicles and macrofollicles is not always stated and classification is subject to the skill, experience and preferences of the cytopathologist. While we minimized this variable by having one cytopathologist (WCF) re-review all cytology reports this itself may bias or alter the ultimate utility of the study to the general population. In the future, molecular markers may help further guide management^[15, 16]. While current molecular platforms have a very high specificity, specimens negative for a genetic alteration may still

be malignant^[16], and repeat biopsy may be useful in identifying these malignancies. Based on our study, after an initial FNAB categorized as AUS/FLUS, clinical variables such as age and gender may help the clinician decide between surgery, repeat biopsy or observation alone. If a decision is made to repeat the FNAB, the repeat FNAB may be performed without a three month wait.

References

1. Cooper DS, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2009; 19(11):1167–214. [PubMed: 19860577]
2. Baloch ZW, et al. 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*. 2008; 36(6):425–37. [PubMed: 18478609]
3. Kim JW, et al. The clinical significance of atypia in thyroid fine-needle aspiration. *Eur Arch Otorhinolaryngol*. 2007; 264(9):1053–7. [PubMed: 17443337]
4. Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. *Thyroid*. 2009; 19(11):1159–65. [PubMed: 19888858]
5. Layfield LJ, et al. Implications of the proposed thyroid fine-needle aspiration category of "follicular lesion of undetermined significance": A five-year multi-institutional analysis. *Diagn Cytopathol*. 2009
6. Wu S, et al. Follicular lesions of the thyroid: A retrospective study of 1,348 fine needle aspiration biopsies. *Diagn Cytopathol*.
7. Faquin WC, Baloch ZW. Fine-needle aspiration of follicular patterned lesions of the thyroid: Diagnosis, management, and follow-up according to National Cancer Institute (NCI) recommendations. *Diagn Cytopathol*.
8. Marchevsky AM, et al. Evidence-based evaluation of the risks of malignancy predicted by thyroid fine-needle aspiration biopsies. *Diagn Cytopathol*. 38(4):252–9. [PubMed: 19813257]
9. Baloch ZW, et al. The National Cancer Institute Thyroid fine needle aspiration state of the science conference: a summation. *Cytojournal*. 2008; 5:6. [PubMed: 18394201]
10. Layfield LJ, et al. Post-thyroid FNA testing and treatment options: a synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. *Diagn Cytopathol*. 2008; 36(6):442–8. [PubMed: 18478610]
11. Yassa L, et al. Long-term assessment of a multidisciplinary approach to thyroid nodule diagnostic evaluation. *Cancer*. 2007; 111(6):508–16. [PubMed: 17999413]
12. Yang J, et al. Fine-needle aspiration of thyroid nodules: a study of 4703 patients with histologic and clinical correlations. *Cancer*. 2007; 111(5):306–15. [PubMed: 17680588]
13. Moon HJ, et al. Can vascularity at power Doppler US help predict thyroid malignancy? *Radiology*. 255(1):260–9. [PubMed: 20308462]
14. Tae HJ, et al. Diagnostic value of ultrasonography to distinguish between benign and malignant lesions in the management of thyroid nodules. *Thyroid*. 2007; 17(5):461–6. [PubMed: 17542676]
15. Nikiforov YE, et al. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. *J Clin Endocrinol Metab*. 2009; 94(6):2092–8. [PubMed: 19318445]
16. Ohori NP, et al. Contribution of molecular testing to thyroid fine-needle aspiration cytology of "follicular lesion of undetermined significance/atypia of undetermined significance". *Cancer Cytopathol*. 118(1):17–23. [PubMed: 20099311]

Synopsis

This is a retrospective review of 203 patients with thyroid FNAB categorized as atypia of undetermined significance at an academic tertiary care center. Treatment algorithms were reviewed and the applicability of waiting for a three month period before repeat biopsy was evaluated. Following an atypical FNAB more than half of the patients were referred for surgery without a repeat biopsy. In patients that had a repeat biopsy, the outcome was not significantly altered by whether it was performed earlier or later than three months.

Figure 1.

Table 1

Risk of malignancy associated with each diagnostic category of a thyroid fine needle aspiration biopsy

Diagnostic Category	Risk of Malignancy (%)
Benign	0–3
Nondiagnostic or Insufficient	1–4
Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance	5–15
Follicular Neoplasm or Suspicious for a	15–30
Follicular Neoplasm	
Suspicious for Malignancy	60–75
Malignant	97–99

Table 2

Comparison of clinical characteristics between the three treatment groups

Characteristic	N = 203	Group I Surgery N = 125 (62%)	Group II Repeat FNA N = 51 (25%)	Group III Follow Up N = 27 (13%)	p-value
Age; mean (+/- SD)		51 (+/-13)	57 (+/-14)	58 (+/-16)	0.006
Sex (%)					
Female		99 (79%)	37 (73%)	26 (96%)	0.04
Male		26 (27%)	14 (26%)	1 (4%)	
History of radiation exposure	N = 19	13 (10%)	5 (26%)	1 (4%)	0.5
History of Hashimoto's	N = 29	17 (38%)	5 (17%)	7 (24%)	0.14

Table 3

Final pathology of patients diagnosed with a malignancy both before and after a repeat biopsy

Final pathology	Surgery without repeat FNAB (Group I) N = 20	Surgery after FNAB (Group II) N = 4
Papillary thyroid carcinoma	10	0
Follicular variant of papillary thyroid carcinoma	4	2
Minimally invasive follicular carcinoma	5	2
Hürthle cell carcinoma	1	0

Table 4

Diagnostic yield of repeat fine needle aspiration biopsies based on time period

Diagnosis on repeat FNAB	Early repeat FNAB (<3 months) n=30	Late repeat FNAB (3 months) n=21
AUS/FLUS	12 (40%)	5 (24%)
Non Diagnostic	3 (10%)	3 (14%)
Benign	11 (36%)	11 (52%)
Suspicious for Follicular Neoplasm	2 (7%)	1 (5%)
Suspicious for Malignancy Malignant	2 (7%)	1 (5%)