Performance of Afirma genomic sequencing classifier and histopathological outcome are associated with patterns of atypia in Bethesda category III thyroid nodules

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BACKGROUND: Data on Afirma's genomic sequencing classifier (GSC) performance in atypia of undetermined significance (AUS) subcategories is limited. This study investigated GSC performance in AUS nodules with architectural atypia (AUS-A), cytological atypia (AUS-C), architectural and cytological atypia (AUS-AC), and predominantly Hürthle cells (AUS-HC). **METHODS:** This study retrieved consecutive thyroid nodules having a recurrent cytologic diagnosis of AUS with qualifiers and a concurrent GSC diagnostic result. All nodules were followed by either surgical intervention or clinical and/or ultrasound monitoring (≥6 months). GSC benign call rate (BCR), rate of histology-proven malignancy, and diagnostic parameters of GSC were calculated for individual AUS subcategories. Statistical analysis was performed using the Fisher exact test. **RESULTS:** A total of 135 AUS nodules fulfilled inclusion criteria, including 79 AUS-A, 9 AUS-C, 29 AUS-AC, and 18 AUS-HC. BCR was 72.2%, 66.7%, 44.8%, and 77.8% in AUS-A, AUS-C, AUS-AC, and AUS-HC, respectively. AUS-A showed a greater BCR than AUS-AC ($p < .05$). All GSC-benign nodules were considered benign on clinical or surgical follow-up. Among GSC-suspicious nodules, histology-proven malignancies represented 4.5% of AUS-A, 0% of AUS-C, 56.3% of AUS-AC, and 25.0% of AUS-HC cases. AUS-AC demonstrated a higher malignant rate compared with AUS-A ($p < .05$). GSC offers 100% NPV and a wide range (5%–56%) of PPV across all AUS subcategories. AUS-AC demonstrated a greater PPV compared with AUS-A ($p < .05$). **CONCLUSION:** BCR of GSC and malignant rates associated with suspicious GSC may differ in various AUS subcategories. GSC-suspicious nodules with both architectural and cytologic atypia are more likely to be malignant. These findings may improve clinical triage and/or management of patients with AUS thyroid nodules. ***Cancer Cytopathol* 2022;130:891-898.**

KEY WORDS: architectural atypia; atypia of undetermined significance; atypia with Hürthle cells; cytologic atypia; genomic sequencing classifier; GSC; molecular testing; thyroid nodules.

INTRODUCTION

Fine-needle aspiration (FNA) has been widely accepted as a major diagnostic test for evaluation of thyroid nodules, aiming to distinguish benign, nonneoplastic nodules that may be managed conservatively with clinical and/or imaging follow-up from neoplastic nodules including malignant entities that require surgical intervention.[1,2] Per the 2015 American Thyroid Association (ATA) management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer, FNA cytology of thyroid nodules should be reported using diagnostic categories outlined in The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC).[3] TBSRTC consists of six categories with each category associated with an implied malignant risk that is linked

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to recommended clinical management. These categories include (I) nondiagnostic, (II) benign, (III) atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), (IV) follicular neoplasm/suspicious for follicular neoplasm (FN/SFN), (V) suspicious for malignancy (SFM), and (VI) malignant. Although TBSRTC definitively categorizes the majority of aspirated thyroid nodules as either a benign, nonneoplastic (60%–70%) or a malignant (5%) nodule and significantly reduces unnecessary surgical intervention, diagnostic challenges remain because approximately one third of the aspirated thyroid nodules fall into indeterminate categories (III, IV, and V), with category III (AUS/FLUS) being the most heterogeneous and troublesome.[4,5] In this regard, it is not uncommon that surgically resected nodules categorized as AUS/FLUS are often ultimately proven to be benign.[3] Previous data from our institution showed histology-proven malignancy in 18%–27% of surgically removed AUS/FLUS nodules whereas the remaining resected nodules were either nonneoplastic (e.g., benign nodular hyperplasia or lymphocytic thyroiditis) or follicular adenomas on histologic assessment.[6,7]

In more recent years, molecular testing incorporated with FNA evaluation has played an important role in further stratification and management of indeterminate thyroid nodules.[8,9] Among several commercially available tests, the Afirma genomic sequencing classifier (GSC) was introduced in 2017, which uses next-generation sequencing, incorporating an ensemble model composed of 12 independent classifiers (10,196 genes with 1115 core genes) and seven other components (parathyroid, medullary thyroid carcinoma, BRAFV600E, RET/PTC and RET/PTC3 detection modules, Hürthle cell index, and Hürthle neoplasm index). Compared with its predecessor, the gene expression classifier (GEC), the newer GSC has demonstrated improved specificity and positive predictive value (PPV) while maintaining a high sensitivity and negative predictive value (NPV).[10,11]

Several studies have reported that AUS thyroid nodules with various patterns of atypia may carry a different malignant risk. Accordingly, AUS with cytological atypia showed a higher malignant risk than AUS with architectural atypia.[12–14] Therefore, the current edition of TBSRTC strongly recommends subcategorization of AUS nodules into one of the following five subgroups—cytologic atypia, architectural atypia, cytologic and architecture atypia, predominance of Hürthle cells, and atypia not otherwise specified (NOS).

It has been reported that thyroid nodules with architectural atypia were more likely to have a benign GEC result, whereas nodules with both cytologic and architecture atypia were mostly likely to be malignant when the GEC result was suspicious.[15] Nevertheless, there is very limited data focusing on the performance of the GSC in subgroups of AUS nodules.

Afirma testing has been used exclusively in our practice for molecular analysis of thyroid FNA specimens since 2013 per the decision of the endocrinologists in our institution. Subclassification of AUS thyroid nodules, along with utilization of GSC in thyroid nodules with a repeat diagnosis of AUS has been a routine practice in our institution since July 2017. This study aimed to investigate the performance of the GSC and evaluate histopathological outcome for subcategories of AUS, namely AUS with architectural atypia (AUS-A), AUS with cytological atypia (AUS-C), AUS with both architectural and cytological atypia (AUS-AC), as well as AUS with predominantly Hürthle cells (AUS-HC).

MATERIALS AND METHODS

This retrospective study was approved by the institutional review board at the University of Michigan in Ann Arbor, Michigan. The study cohort included consecutive thyroid nodules with repeated AUS diagnosis accompanied by a "benign" or "suspicious" result of Afirma GSC testing during a 4-year period (July 2017–June 2021). Diagnostic criterion of AUS subcategories was similar to what has been previously described by Baca et al.[15] An aspirate was interpreted as AUS-A when it had bland-appearing follicular cells arranged mainly in microfollicles and/or trabeculae, but was insufficient for a diagnosis of FN/SFN. An aspirate was categorized as AUS-C when it showed follicular cells mainly exhibiting nuclear features (powdery chromatin, irregular nuclear membrane, and intranuclear grooves and/or pseudoinclusions) concerning for papillary thyroid carcinoma (PTC), but was neither quantitatively nor qualitatively sufficient for a diagnosis of suspicious PTC nor positive PTC. A diagnosis of AUS-AC was rendered when an aspirate had a combination of both architectural and cytological atypia described above. AUS-HC was applied to a specimen that was comprised almost exclusively of Hürthle cells but was

insufficient for a diagnosis of Hürthle cell type of FN/SFN.

All of the aspirated nodules were followed by either subsequent surgical intervention or at least 6 months of clinical and/or ultrasound monitoring. Nodules with an Afirma result of "nondiagnostic" (due to inadequate sampling) and nodules lacking both surgical follow-up and appropriate clinical and/or ultrasound monitoring were excluded from the study.

Ultrasound-guided thyroid FNAs were performed by radiologists and/or endocrinologists with cytology-assisted rapid on-site adequacy assessment. Two conventional smears were made for each pass. One smear was air-dried and stained with Diff-Quik protocol to be evaluated immediately for specimen adequacy whereas the other smear was fixed with Sprayfix and later stained with a Papanicolaou stain. The needle was then rinsed in Cytolyt solution for making a ThinPrep slide and/or a cell block. Two dedicated passes were simultaneously collected into the Afirma-provided fixative vial for thyroid nodules that had a previous diagnosis of AUS. FNA specimens were then assessed by a subspecialty board certified cytopathologist and diagnoses were reported using TBSRTC system. When a recurrent diagnosis of AUS was rendered, the aforementioned pre-collected samples were sent to Veracyte's CLIA laboratory (South San Francisco, CA) for Afirma GSC testing.

The following information from individual patients were collected and recorded: age, gender, size of thyroid nodule, modality of follow-up (surgical vs. nonsurgical), corresponding histologic diagnosis (if surgically treated), as well as stability (lack of change in nodule size and characteristics) of nonsurgically removed nodules during the period of at least 6 months of clinical and/or ultrasound monitoring. Based on subsequent ultrasound findings and/or clinical notes/observation, the nodules with stable and benign characteristics were considered benign (clinical benign diagnosis).

Benign call rate (BCR) of GSC, rate of histology-proven malignancy (ROM) associated with a suspicious GSC result, and diagnostic parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated for each of the AUS subcategories as follows:

- BCR = number of GSC-benign nodules/total number of nodules with GSC testing;
- ROM = number of histology proven malignancies/number of suspicious GSC results;
- Sensitivity = number of GSC-suspicious nodules with histology-proven malignancy (true-positive)/number of all histology proven malignant nodules (true-positive + false-negative);
- Specificity = number of GSC-benign nodules with a subsequent surgical and/or clinical benign diagnosis (true-negative)/number of all benign nodules (true-negative + false-positive);
- PPV = true-positive/all GSC-suspicious nodules (true-positive + false-positive);
- NPV = true-negative/all GSC-benign nodules (true-negative + false-negative); and
- Diagnostic accuracy = (true-positive + true-negative)/total number of nodules.

Fisher exact test for categorical variables and Student t-test for continuous variables were performed using Social Science Statistics (https://www.socscistatistics.com/tests). Statistical significance was defined as a two-tailed p value of <.05 for all analysis.

RESULTS

Study cohort

A total 3572 thyroid FNAs were performed during the 4-year study period (July 2017–June 2021). Of which, 745 (745 of 3572 = 20.9%) were initially categorized as AUS. A repeat FNA was performed on 226 (226 of 745 = 30.3%) AUS nodules. Among which, 179 (179 of 226 = 79.2%) remained as AUS with one of the qualifiers (AUS-A, AUS-C, AUS-AC, or AUS-HC), 45 (45 of 226 = 19.9%) were recategorized as benign, and the remaining two were interpreted as suspicious for follicular neoplasm and suspicious for medullary carcinoma, respectively. Of the 179 nodules with a repeat AUS diagnosis, GSC testing was performed in 140 nodules. Excluding the five nodules that either had a nondiagnostic GSC result or lack of follow-up, the study consisted of a total of 135 thyroid nodules that fulfilled the aforementioned inclusion criteria. Table 1 summarizes the patient demographic and size distribution of thyroid nodules across the different subcategories. The most common pattern of atypia was AUS-A (79 of 135 = 58.5%) followed by AUS-AC (29 of 135 = 21.5%), AUS-HC (18 of 135 = 13.3%), and AUS-C (9 of 135 = 6.7%). Female

TABLE 1. Clinical characteristics of the study cohort

Characteristic	AUS-A	AUS-C	AUS-AC	AUS-HC	Total
No. of nodules	79	9	29	18	135
Sex					
F	52	7	18	13	90
M	27	2	11	5	45
Patient age, year (range)	56 (19–82)	57 (32–71)	53 (30–80)	52 (27–80)	55 (19–82)
Nodule size (cm)					
1–3	66	6	25	15	112
>3	13	3	4	3	23

Abbreviations: A, architectural atypia; AC, architectural and cytological atypia; AUS, atypia of undetermined significance; C, cytological atypia; F, female; HC, Hürthle cell dominant; M, male.

TABLE 2. Clinical features and relation to GSC results

Variable	GSC benign	GSC suspicious	Proportion of GSC benign, %	*p*
Total	90	45	66.7	
Sex				
F	59	31	65.5	
M	31	14	68.9	.85
Nodule size (cm)				
<3 cm	72	40	64.3	
>3 cm	18	5	78.2	.23

Abbreviations: F, female; GSC, genomic sequencing classifier; M, male.

predominance occurred in each of the AUS subcategories whereas the patients in different subcategories had a similar age range. Over 80% of the nodules measured ≤3 cm in greatest dimension, representing 83.5% (66 of 79) of AUS-A, 66.7% (6 of 9) of AUS-C, 86.2% (25 of 29) of AUS-AC, and 83.3% (15 of 18) of AUS-HC.

BCR and follow-up of GSC-benign nodules

GSC interpreted 90 of 135 AUS nodules as benign with an overall BCR of 66.7%. The difference in BCR between female and male patients was not significant. Similarly, BCR in nodules ≤3 cm did not differ significantly from that of nodules >3 cm (Table 2). BCR reached over 70% in both AUS-HC (14 of 18 = 77.8%) and AUS-A (57 of 79 = 72.2%) subcategories, followed by 66.7% (6 of 9) in AUS-C and 44.8% (13 of 29) in AUS-AC subcategories. The difference in BCR was statistically significant when comparing AUS-HC versus AUS-AC ($p = .03$), as well as AUS-A versus AUS-AC ($p = .01$) (Figure 1). The majority of the GSC-benign nodules in the AUS-A, AUS-AC, and AUS-HC subcategories, as well as all GSC-benign nodules in the AUS-C subcategory were considered stable and benign following at least 6 months of clinical and/or ultrasound monitoring. Of all GSC-benign nodules, 12 AUS-A nodules, three AUS-AC nodules, and three AUS-HC nodules underwent surgical resection and were proved to be benign. Histopathological examination revealed mainly nonneoplastic changes with predominantly nodular hyperplasia and rarely Hashimoto's thyroiditis. One follicular adenoma, which included the presence of Hürthle cells, was found in each of the AUS-A, AUS-AC, and AUS-HC subcategories (Table 3).

Follow-up of GSC-suspicious nodules

Of the 135 AUS nodules, GSC interpreted 45 nodules as suspicious, representing less than one third of nodules subcategorized as AUS-A, AUS-C, or AUS-HC. Over 50% (16 of 29) of AUS-AC nodules had a suspicious GSC result. Surgical intervention was implemented in all GSC-suspicious nodules subcategorized as AUS-A or AUS-C, as well as in most GSC-suspicious nodules subcategorized as AUS-AC (15 of 16 = 93.8%) or AUS-HC (3 of 4 = 75.0%). One patient in each of the latter two groups (AUS-AC and AUS-HC) declined surgical treatment. Histology-proven malignancy presented in more than 50% (9 of 16) of AUS-AC nodules, including eight papillary thyroid carcinomas and one medullary thyroid carcinoma. On the other hand, only one follicular/Hürthle cell carcinoma was identified in each of the AUS-A and AUS-HC subcategories, and none of the AUS-C nodules were malignant on histologic assessment. AUS-AC nodules demonstrated a significantly greater ROM compared with AUS-A nodules ($p = .0003$). Additionally, AUS-A nodules with suspicious GSC results most commonly

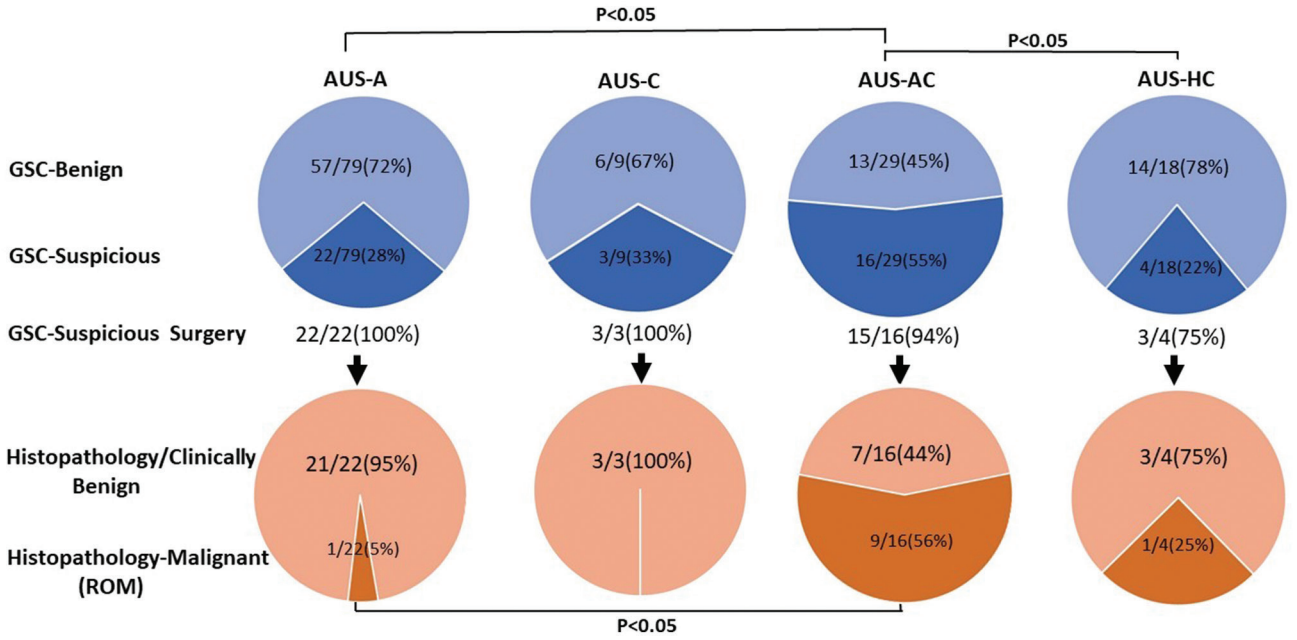

Figure 1. GSC results and histopathologic outcomes in thyroid nodules with different subtypes of atypia. The difference in BCR was statistically significant when comparing AUS-HC versus AUS-AC (p = .03), as well as AUS-A versus AUS-AC (p = .01). AUS-AC nodules demonstrated a significantly greater ROM compared with AUS-A nodules (p = .0003). A indicates architectural atypia; AC, architectural and cytological atypia; AUS, atypia of undetermined significance; BCR, benign call rate; C, cytological atypia; GSC, genomic sequencing classifier; HC, Hürthle cell dominant; ROM, rate of malignancy.

TABLE 3. Clinical follow-up and histopathological diagnoses of GSC benign nodules

Diagnosis	AUS-A	AUS-C	AUS-AC	AUS-HC	Total
GSC-benign	57	6	13	14	90
Clinically stable	45	6	10	11	72
Surgically treated	10	2	3	3	18
Nodular hyperplasia	9		2	2	13
Hashimoto thyroiditis		2			2
Follicular adenoma	1		1		2
Hürthle cell adenoma				1	1

Abbreviations: AUS, atypia of undetermined significance; GSC, genomic sequencing classifier.

proved to either be a follicular adenoma ($n = 7$) or noninvasive follicular neoplasm with papillary nuclear features (NIFTP) ($n = 2$). Less than 20% (3 of 16) of AUS-AC nodules and 25% (1 of 4) of AUS-HC nodules were derived from a follicular/Hürthle cell adenoma (Figure 1 and Table 4).

Diagnostic performance of GSC testing in AUS subcategories

As seen in Table 5, GSC testing offers 100% sensitivity and NPV across most or all AUS subcategories. Both specificity and diagnostic accuracy reached over 65% in all subcategories. PPV showed a wide range (5%–56%) across the various subcategories. In this regard, PPV in AUS-AC nodules is significantly greater than that of AUS-A nodules ($p = .0005$).

DISCUSSION

Since the validation study of GSC for the preoperative evaluation of cytologically indeterminate thyroid nodules,[16] there have been several published studies reporting real-world comparison of the performance between GSC versus GEC in thyroid nodules categorized into Bethesda category III or IV.[2,17–20] A meta-analysis of these aforementioned studies was published during 2019–2020[11] as well as the several studies in 2021 have demonstrated similar benefits of GSC, such as elevated BCR and improved diagnostic performance, particularly with regards

TABLE 4. Clinical follow-up and histopathological diagnoses of GSC suspicious nodules

Diagnosis	AUS-A	AUS-C	AUS-AC	AUS-HC	Total
GSC-suspicious	22	3	16	4	45
Clinically stable			1	1	2
Surgically treated	22	3	15	3	43
Benign histopathology					
Nodular hyperplasia	12	3	3	1	19
Follicular adenoma	7		3		10
Hürthle cell adenoma				1	1
NIFTP[a]	2				2
Malignant histopathology					
Papillary thyroid carcinoma			6		6
PTC (follicular variant)			2		2
Follicular carcinoma	1				1
Hürthle cell carcinoma				1	1
Medullary carcinoma			1		1

Abbreviations: A, architectural atypia; AC, architectural and cytological atypia; AUS, atypia of undetermined significance; C, cytological atypia; HC, Hürthle cell dominant; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features.
[a]NIFTP was grouped with follicular/Hürthle cell adenoma rather than malignancy based on the currently available consensus that NIFTP has a good prognosis and can be managed as follicular/Hürthle cell adenoma.

TABLE 5. Diagnostic performance of Afirma GSC by subtype of atypia

Column1	AUS-A, %	AUS-C, %	AUS-AC, %	AUS-HC, %	All AUS, %
Sensitivity	100		100	100	100
Specificity	73	67	65	82	73
PPV	5		56	25	24
NPV	100	100	100	100	100
Diagnostic accuracy	73	67	76	83	75

Abbreviations: A, architectural atypia; AC, architectural and cytological atypia; AUS, atypia of undetermined significance; C, cytological atypia; GSC, genomic sequencing classifier; HC, Hürthle cell dominant; NPV, negative predictive value; PPV, positive predictive value.

to specificity and PPV.[10,21,22] To the best of our knowledge, studies focused on the performance of GSC in individual AUS subcategories are lacking. The current study accordingly investigated the performance of GSC and histopathological outcome in individual subcategories of AUS including AUS-A, AUS-C, AUS-AC, and AUS-HC.

In the current study, GSC showed an overall BCR of 66.7% for all AUS nodules, which is in line with BCRs reported previously (61.0%–80.6%).[2,18–20,22] When subtyping AUS, BCR ranged from 44.8% (AUS-AC) to over 70% (72.2% for AUS-A and 77.8% for AUS-HC), demonstrating a significant difference in BCR in AUS subcategories. Furthermore, the current study revealed a histology-proven malignancy in 24.4% of all AUS nodules with a suspicious GSC result, corresponding with an overall PPV of 24%. This PPV appears to be lower than that of previous studies (57% and 52%).[2,22] However, individual AUS subcategories of the current study showed marked variation in rates of subsequent histology-proven malignancy and PPV. In this regard, histology-proven malignancies represented 56.3% of AUS-AC, followed by 25% of AUS-HC and 4.5% of AUS-A, whereas all three AUS-C GSC suspicious nodules proved to be nodular hyperplasia. Thus, a wide range of PPV was evident (0%–56%). AUS-AC demonstrated the greatest PPV (56%), which is significantly higher than that of AUS-A. Taken together, the difference in overall BCR and PPV between our study and the previously reported studies may result from variations in distribution of individual AUS subcategories. Awareness of the potential difference of GSC performance in different AUS subcategories may aid in more appropriate triage and management of patients with AUS nodules.

GSC has been reported to prompt a greater BCR (60%–80%) and potentially minimize unnecessary surgeries in Hürthle cell predominant nodules.[16–18] In the current study, BCR reached 77.8% in AUS-HC. Similar to other GSC-benign nodules in other AUS subcategories, GSC-benign nodules in the AUS-HC subcategory were considered benign on surgical and clinical follow-up.

Baca et al.[15] conducted a study to compare GEC performance across different AUS subcategories including AUS-A, AUS-C, and AUS-AC. In their study, the highest BCR was seen in AUS-A (65%), followed by

AUS-C (49%) and AUS-AC (38%). Within their surgically resected GEC-suspicious nodules, histology-proven malignancy was documented in 57% of AUS-AC, 45% of AUS-C, and 19% of AUS-A.[15] In our study, GSC showed a similar trend in BCR (72.2%, 66.7%, and 44.8% in AUS-A, AUS-C, and AUS-AC, respectively). With regard to the surgically resected GSC-suspicious nodules, AUS-AC also showed the greatest rate of histology-proven malignancy (60%). A total of 5% of AUS-A nodules proved to be malignant, whereas all AUS-C nodules showed nodular hyperplasia. It is noteworthy to mention that despite the earlier GEC study and our current GSC study containing a similar proportion of AUS-A nodules, our study had less case numbers in terms of AUS-C (9 vs. 55), GSC-suspicious AUS-C (3 vs. 28), and surgically removed GSC-suspicious AUS-C (3 vs. 22) nodules. It seems that AUS-C alone was less common than AUS-AC in our institution. These variations may potentially contribute to the low incidence of histology-proven malignancy for AUS-C nodules. However, it would be difficult to draw a definitive conclusion due to the limited case cohort.

One of the limitations of the current study is that GSC testing was routinely applied to those thyroid nodules that were categorized into the TBSRTC category III (AUS) at a second time. It is unknown if outcomes of GSC testing may have varied if these nodules were to have received GSC testing at the time of their initial diagnosis of AUS. Although focusing on GEC, Baca et al.[15] claimed no difference was observed in BCR or in the malignancy rate when comparing their patient's nodules with a repeat indeterminate cytology result versus nodules that had a single indeterminate cytology result.[15] On the other hand, Nishino et al.[23] found that GEC performed on nodules with a repeat indeterminate cytology result would decrease surgical rates for histologically benign nodules while missing rare, low-risk neoplasms (i.e., NIFTP). These discrepant conclusions highlight the importance of further study in this realm to optimize patient management pathways.

The limitations of the current study also include a relatively small case cohort in each of the AUS subcategories and a short follow-up period (minimum of 6 months) for the clinical benign nodules. With regard to the latter, it is worth mentioning that many indolent low-grade thyroid carcinomas will not demonstrate clinically malignant features for many years (over 10 years).

Our study demonstrated that BCR of GSC and the malignant rate associated with a suspicious GSC result may differ in various AUS subcategories. AUS-A showed a significantly greater BCR than AUS-AC, whereas AUS-AC demonstrated a significantly higher malignant rate compared with AUS-A. These findings may guide more appropriate clinical triage and/or improve management of patients with AUS thyroid nodules.

AUTHOR CONTRIBUTIONS

Xiaobing Jin: Acquired, analyzed, and interpreted data and resource materials, drafted the manuscript and contributed significant revisions on subsequent drafts, contributed to final approval of version to be published, and agrees to be accountable for all aspects of the work in ensuring questions related to accuracy or integrity of any part of the work. **Madelyn Lew:** Contributed revisions for intellectual content, contributed to final approval of version to be published, and agrees to be accountable for all aspects of the work in ensuring questions related to accuracy or integrity of any part of the work. **Liron Pantanowitz:** Contributed revisions for intellectual content, contributed to final approval of version to be published, and agrees to be accountable for all aspects of the work in ensuring questions related to accuracy or integrity of any part of the work. **Brian Smola:** Contributed revisions for intellectual content, contributed to final approval of version to be published, and agrees to be accountable for all aspects of the work in ensuring questions related to accuracy or integrity of any part of the work. **Xin Jing:** Designed concept of article, acquired/analyzed/interpreted data and resource materials, drafted the manuscript and contributed significant revisions on subsequent drafts, contributed to final approval of version to be published, and agrees to be accountable for all aspects of the work in ensuring questions related to accuracy or integrity of any part of the work.

CONFLICTS OF INTEREST

The authors made no disclosures.

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