Clinical Performance of the Afirma Genomic Sequencing Classifier (GSC) in Pediatric Patients With Cytologically Indeterminate Thyroid Nodules

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INTRODUCTION

- Cytologically indeterminate thyroid nodules (ITN) in pediatrics carry a higher risk of malignancy (~30-60%) compared with adult ITN (13-34%).
- The 2015 ATA pediatric thyroid nodule guidelines recommend surgical resection for ITN.[1]
- The Afirma GSC is an exome-enriched RNAseq assay that was validated in patients > 21 yrs with a 96% negative predictive value (NPV) for malignancy when benign (GSC-B) in lesions with indeterminate cytology, allowing patients to avoid unnecessary thyroid surgery.[2]
- The goal of this study was to evaluate the performance of the Afirma GSC in pediatric patients with ITN.

METHODS

- Forty-nine ITN from patients < 21 yrs sent for Afirma GSC testing had histopathology or clinical follow-up data collected.
  - These outcomes were collected on consecutive patients and were not known prior to inclusion in the study.
- Afirma GSC ensemble classifier and Xpression Atlas (XA – the GSC molecular variant and fusion panel) data were analyzed for all samples (though XA data is not reported clinically for GSC-B cases).
- For Afirma GSC-B cases that *avoided* surgery, a two-year clinical follow-up with exam and/or ultrasound was a surrogate for a true negative (TN) result.

RESULTS

- Nine males and 40 females (ages 12-20 yrs, median 18.5 [IQR 17.3-19.8]) had 30 GSC-B and 19 GSC-suspicious (S) cases from nodules ranging from < 1cm to > 7cm in size (Table 1 and Figure 1).
- Of GSC-S cases that were resected, histopathology determined that 14 (73.7%) were malignant (true positive, TP) and five (26.3%) were benign (false positive, FP) (Table 1).
- All GSC-B cases were either histologically (n=9) or clinically benign (TN) (Table 1).
- All 14 malignancies were GSC-S (sensitivity 100% [95% CI 77-100%]); 30/35 clinically or histologically benign cases were GSC-B (specificity 86% [95% CI 70-95%]). The NPV for a GSC-B result was 100% [95% CI 88-100%] (Figure 2).
- Genomic alterations were not detected by XA in the 30 Afirma benign samples and there was a *DICER1* alteration in one of the five false positive lesions (Table 2).
- Among the 14 TP samples, there were 7 papillary thyroid carcinomas (PTC; n=1 with *FGFR2::VCL* fusion and n=1 *PPP1R21::ALK* fusion (Fig 3)); 1 oncocytic carcinoma (*PAX8::PPARG* fusion); 1 NIFTP; 2 infiltrative follicular subtype of PTC; and 3 follicular thyroid carcinomas (n=1 with *PAX8::PPARG* fusion, n=2 with *NRAS*p.Q61K) (Table 2).
  - The *FGFR2::VCL* fusion was recently reported in 4 thyroid tumor samples from a cohort of 5030 sequenced thyroid malignancies[3] and was reported in a 21-year-old with BIII cytology who had a PTC on final histopathology.[4]
  - The *PPP1R21::ALK* fusion has previously been reported in 6 thyroid tumor samples from the Afirma DB without histologic correlate.[5]
  - The *PPP1R21::ALK* fusion has been described when testing a resected tumor in a 12-year-old female with a classic PTC and T2N1b histopathology findings that had a Bethesda VI cytology result.[6]
  - To our knowledge, this is the first report of detection of the *PPP1R21::ALK* fusion in a pre-operative thyroid tumor specimen with Bethesda III cytology and a histologic correlate.
    - Figure 3 shows a representative schematic of the *FGFR2::VCL* (3A) and *PPP1R21::ALK* (3B) fusions.

TABLE 1

Study cohort data

Xpression Atlas: empty cells are negative for a variant or fusion
Surgery Type: for Afirma benign cases, empty cells at surgery type and beyond indicate the patient had at least two years of clinical follow up with no surgery
TP: true positive, **FP:** false positive, **TN:** true negative, **FN:** false negative relative to the Afirma result

NH: nodular hyperplasia
FA: follicular adenoma
PTC: papillary thyroid carcinoma
OC: oncocytic carcinoma
NIFTP: noninvasive follicular thyroid neoplasm with papillary-like features
IFVPTC: infiltrative follicular variant PTC
FTC: follicular thyroid carcinoma
OA: oncocytic adenoma

Sex	Age	Nodule Size	Bethesda Category	Afirma GSC Result	Xpression Atlas Result	Surgery Type	(B)enign or (M)alignant	Histopathology	Size (cm) at Histology	TP/FP/TN/FN
F	18.07	1.4	AUS/FLUS (Bethesda III)	Benign						TN
F	20.71	1	AUS/FLUS (Bethesda III)	Benign						TN
F	18.62	2.1	AUS/FLUS (Bethesda III)	Benign						TN
F	18.99	2.6	AUS/FLUS (Bethesda III)	Benign		(unstated - path dx from clinic notes)	B			TN
F	17.67	2.5	AUS/FLUS (Bethesda III)	Benign						TN
M	15.8	1.6	AUS/FLUS (Bethesda III)	Benign		(unstated - path dx from clinic notes)	B	NH	unstated	TN
F	14.75	3.7	FN/SFN (Bethesda IV)	Benign						TN
F	17.72	5.7	AUS/FLUS (Bethesda III)	Benign		(unstated - path dx from clinic notes)	B	FA	unstated	TN
F	17.62	5.1	AUS/FLUS (Bethesda III)	Benign						TN
F	19.37	3.2	AUS/FLUS (Bethesda III)	Benign						TN
F	19.63	1.4	AUS/FLUS (Bethesda III)	Benign						TN
F	20.83	1.5	AUS/FLUS (Bethesda III)	Benign						TN
M	17.6	0.95	AUS/FLUS (Bethesda III)	Benign		(unstated - path dx from clinic notes)	B			TN
F	18.53	1.1	FN/SFN (Bethesda IV)	Benign						TN
M	14.48	3.6	AUS/FLUS (Bethesda III)	Benign		Right lobectomy	B			TN
M	20.06	5.1	FN/SFN (Bethesda IV)	Benign		Right lobectomy	B	FA	4.0	TN
F	17.25	7.3	AUS/FLUS (Bethesda III)	Benign		Left lobectomy	B	FA	4.6	TN
M	18.57	1	AUS/FLUS (Bethesda III)	Benign						TN
F	20.97	5	FN/SFN (Bethesda IV)	Benign		Total thyroidectomy	B	FA	3.5	TN
F	20.58	3.9	FN/SFN (Bethesda IV)	Benign						TN
F	20.12	2.29	FN/SFN (Bethesda IV)	Benign						TN
F	19.83	2.1	FN/SFN (Bethesda IV)	Benign						TN
F	16.75	2.2	AUS/FLUS (Bethesda III)	Benign						TN
F	18.37	1.2	FN/SFN (Bethesda IV)	Benign						TN
F	19.83	2	AUS/FLUS (Bethesda III)	Benign						TN
M	20.89	2.8	AUS/FLUS (Bethesda III)	Benign						TN
F	18.67	1.2	FN/SFN (Bethesda IV)	Benign						TN
F	19.81	1.9	AUS/FLUS (Bethesda III)	Benign						TN
M	17.97	2	AUS/FLUS (Bethesda III)	Benign		(unstated - path dx from clinic notes)	B		unstated	TN
F	15.26	1.6	AUS/FLUS (Bethesda III)	Benign						TN
F	17.14	1.8	AUS/FLUS (Bethesda III)	Suspicious		Left lobectomy	M	PTC	0.8	TP
M	19.84	7.5	FN/SFN (Bethesda IV)	Suspicious	*PAX8::PPARG*	Left lobectomy	M	OC	6.5	TP
F	20.31	2.8	FN/SFN (Bethesda IV)	Suspicious		Right lobectomy	M	NIFTP	2.9	TP
F	19.77	1.8	AUS/FLUS (Bethesda III)	Suspicious	*FGFR2::VCL*	Right lobectomy	M	PTC	1.4	TP
F	20.39	1.1	AUS/FLUS (Bethesda III)	Suspicious	*PPP1R21::ALK*	Left lobectomy	M	PTC	1.5	TP
F	16.87	1	AUS/FLUS (Bethesda III)	Suspicious		Left lobectomy	M	IFVPTC	0.88	TP
F	17.75	1.1	AUS/FLUS (Bethesda III)	Suspicious		Total thyroidectomy	M	PTC	1.9	TP
F	18.47	4	AUS/FLUS (Bethesda III)	Suspicious	*PAX8::PPARG*	Total thyroidectomy	M	FTC	4.1	TP
F	17.4	1.2	AUS/FLUS (Bethesda III)	Suspicious		Total thyroidectomy	M	IFVPTC	0.9	TP
F	12.76	0.9	FN/SFN (Bethesda IV)	Suspicious		Total thyroidectomy	M	PTC	1.2	TP
F	9.89	1.9	AUS/FLUS (Bethesda III)	Suspicious		Total thyroidectomy	M	PTC	2.2	TP
F	20.53	1.35	AUS/FLUS (Bethesda III)	Suspicious	*NRAS p.Q61K*	Hemi then completion thyroidectomy	M	FTC	1.5	TP
F	13.51	1.1	FN/SFN (Bethesda IV)	Suspicious		Total thyroidectomy	M	PTC	1.1	TP
F	19.9	2.4	AUS/FLUS (Bethesda III)	Suspicious	*NRAS p.Q61K*	Right lobectomy	M	FTC	2.6	TP
F	19.78	3.2	FN/SFN (Bethesda IV)	Suspicious		Right lobectomy	B	OA	2.5	FP
M	19.07	1.8	AUS/FLUS (Bethesda III)	Suspicious		(unstated - path dx from clinic notes)	B		unstated	FP
F	17.13	1.5	AUS/FLUS (Bethesda III)	Suspicious		(unstated - path dx from clinic notes)	B		unstated	FP
F	16.78	1.75	AUS/FLUS (Bethesda III)	Suspicious		Total thyroidectomy	B	FA	1.8	FP
F	18.42	1.74	AUS/FLUS (Bethesda III)	Suspicious	*DICER1 p.E1813Q*	Lobectomy (side unspecified in notes)	B		unstated	FP

FIGURE 1

Distribution of nodule size (cm) in the cohort. ']' indicates inclusion of number and '(' indicates exclusion.

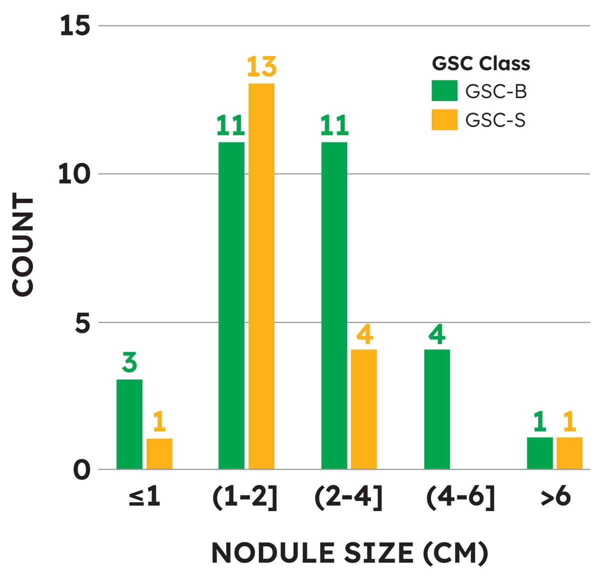

FIGURE 3

Schematic of the *FGFR2::VCL* and *PPP1R21::ALK* fusions

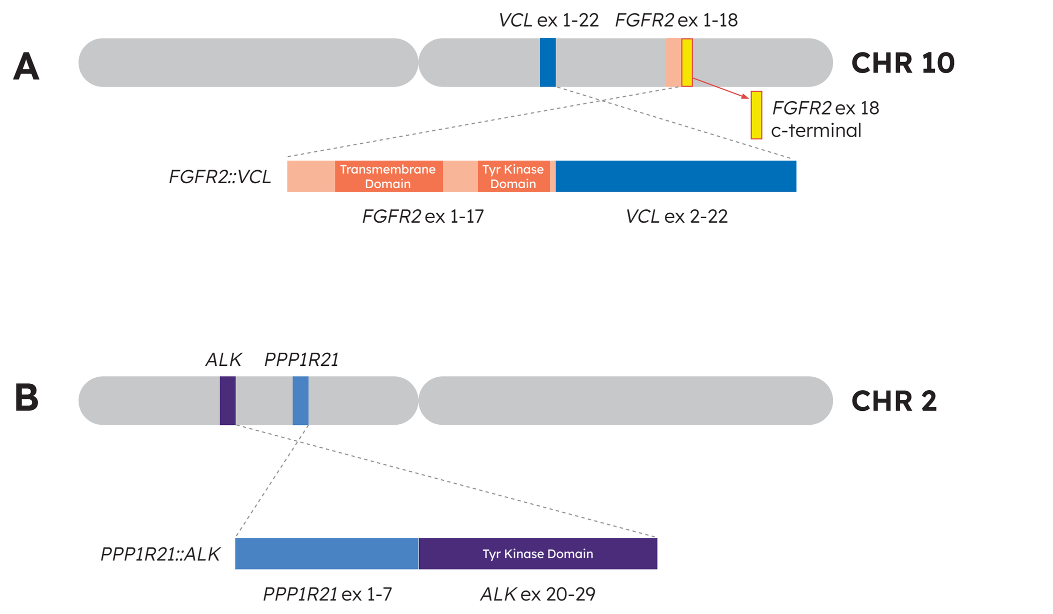

FIGURE 2

Afirma test performance characteristics

	Benign	Malignant
GSC-S	FP 5	TP 14
GSC-B	TN 30	FN 0

HISTOLOGY

Sensitivity: 100% [95% CI 76.8-100]
Specificity: 85.7% [95% CI 69.7-95.2]
PPV: 73.7% [95% CI 55.4-86.3]
NPV: 100% [95% CI 88.4-100]

TABLE 2

Genomic alterations detected by the Afirma Xpression Atlas

PTC: papillary thyroid carcinoma **OC:** oncocytic carcinoma **FTC:** follicular thyroid carcinoma

Sex	Age	Nodule Size	Bethesda Category	Xpression Atlas Result	Surgery Type	(B)enign or (M)alignant	Histopathology	Size (cm) at Histology	TP/FP/TN/FN
M	19.84	7.5	FN/SFN (Bethesda IV)	*PAX8::PPARG*	Left lobectomy	M	OC	6.5	TP
F	19.77	1.8	AUS/FLUS (Bethesda III)	*FGFR2::VCL*	Right lobectomy	M	PTC	1.4	TP
F	20.39	1.1	AUS/FLUS (Bethesda III)	*PPP1R21::ALK*	Left lobectomy	M	PTC	1.5	TP
F	18.47	4	AUS/FLUS (Bethesda III)	*PAX8::PPARG*	Total thyroidectomy	M	FTC	4.1	TP
F	20.53	1.35	AUS/FLUS (Bethesda III)	*NRAS p.Q61K*	Hemi then completion thyroidectomy	M	FTC	1.5	TP
F	19.9	2.4	AUS/FLUS (Bethesda III)	*NRAS p.Q61K*	Right lobectomy	M	FTC	2.6	TP
F	18.42	1.74	AUS/FLUS (Bethesda III)	*DICER1 p.E1813Q*	Lobectomy (side unspecified in notes)	B		unstated	FP

CONCLUSION

- In older adolescents with ITN, the Afirma GSC showed excellent performance when defining a true negative result by histology or clinical follow-up.
- Our findings indicate that an Afirma GSC-B result appears reassuring and may allow for a more conservative management strategy in younger patients with ITN.
- The exome-enriched RNAseq Afirma GSC platform additionally allows for the discovery of rare and targetable alterations such as *ALK* and *FGFR2* fusions. More data is required to fully understand the clinical significance of pediatric thyroid tumors harboring these rare fusions.

REFERENCES

1. Francis GL, Waguespack SG, Bauer AJ, Angelos P, Benvenga S, Cerutti JM, Dinauer CA, Hamilton J, Hay ID, Luster M, Parisi MT, Rachmiel M, Thompson GB, Yamashita S; American Thyroid Association Guidelines Task Force. Management Guidelines for Children with Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2015 Jul;25(7):716-59. doi: 10.1089/thy.2014.0460. PMID: 25900731.
2. Patel KN, Angell TE, Babiarz J, Barth NM, Blevins T, Duh QY, Ghossein RA, Harrell RM, Huang J, Kennedy GC, Kim SY, Kloos RT, LiVolsi VA, Randolph GW, Sadow PM, Shanik MH, Sosa JA, Traweek ST, Walsh PS, Whitney D, Yeh MW, Ladenson PW. Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules. JAMA Surg. 2018 Sep 1;153(9):817-824. doi: 10.1001/jamasurg.2018.1153. PMID: 29799911.
3. Sabbagh MF, Janovitz T, Dias-Santagata D, Siegmund S, Nardi V, Wirth LJ, Randolph GW, Lennerz JK, Decker B, Nose V, Alzumaili BA, Faquin WC, Barletta JA, Le LP, Iafrate AJ, Sadow PM, Fisch AS. FGFR Alterations in Thyroid Carcinoma: A Novel Class of Primary Drivers with Significant Therapeutic Implications and Secondary Molecular Events Potentially Mediating Resistance in Thyroid Malignancy. Thyroid. 2024 Sep;34(9):1137-1149. doi: 10.1089/thy.2024.0216. Epub 2024 Aug 7. PMID: 39003525.
4. Munoz-Zuluaga CA, Heymann JJ, Solomon JP, Patel A, Siddiqui MT, Scognamiglio T, Gokozan HN. Use of the Afirma Xpression Atlas for cytologically indeterminate, Afirma Genomic Sequencing Classifier suspicious thyroid nodules: Clinicopathologic analysis with postoperative molecular testing. Am J Clin Pathol. 2024 May 2;161(5):463-468. doi: 10.1093/ajcp/aqad169. PMID: 38104250.
5. Hu MI, Waguespack SG, Dosiou C, Ladenson PW, Livhits MJ, Wirth LJ, Sadow PM, Krane JF, Stack BC, Zafereo ME, Ali SZ, Weitzman SP, Hao Y, Babiarz JE, Kennedy GC, Kloos RT. Afirma Genomic Sequencing Classifier and Xpression Atlas Molecular Findings in Consecutive Bethesda III-VI Thyroid Nodules. J Clin Endocrinol Metab. 2021 Jul 13;106(8):2198-2207. doi: 10.1210/clinem/dgab304. PMID: 34009369.
6. Panebianco F, Nikitski AV, Nikiforova MN, Kaya C, Yip L, Condello V, Wald AI, Nikiforov YE, Chiosea SI. Characterization of thyroid cancer driven by known and novel ALK fusions. Endocr Relat Cancer. 2019 Nov;26(11):803-814. doi: 10.1530/ERC-19-0325. PMID: 31539879.