"Molecular Analysis of Non-Diagnostic (B-I) Thyroid Nodule Cytology Samples Using Combined Mutational Detection and microRNA Classifier Test"

Sydney Finkelstein,[1,2] Gyanendra Kumar,[1] Venkata Arun Timmaraju,[1,2] Kenny Ablordeppey,[1] Mahmoud Shalaby,[3] Rizwan Aslam,[3] Keith Haugh,[2] Christina Narick,[2] Alidad Mireskandari,[1] and Emad Kandil[3]

[1]Interpace Diagnostics Lab, 2 Church Street South, Suite B05, New Haven, CT 06519 [2]Interpace Diagnostics Inc., 2515 Liberty Avenue, Pittsburgh, PA 15222

[3]Department of Surgery, Tulane University School of Medicine, New Orleans, LA 70112

Background

- Non-diagnostic cytology (Bethesda I) occurs in 5%-20% of thyroid nodule fine needle aspirates (FNAs),[1] often resulting in repeat FNA procedures, inconvenience for patients, and increased healthcare costs
- Interpace Diagnostics previously presented data showing that molecular testing can provide useful diagnostic information for the majority of samples with non-diagnostic (B-I) cytology from a dedicated pass collected in RNARetain[2]
- We hypothesized that B-I cases could potentially be addressed by molecular analysis utilizing a combination testing approach with a mutational panel (ThyGeNEXT®) along with a microRNA classifier (ThyraMIR®)

Aims

The aims of this study are to:

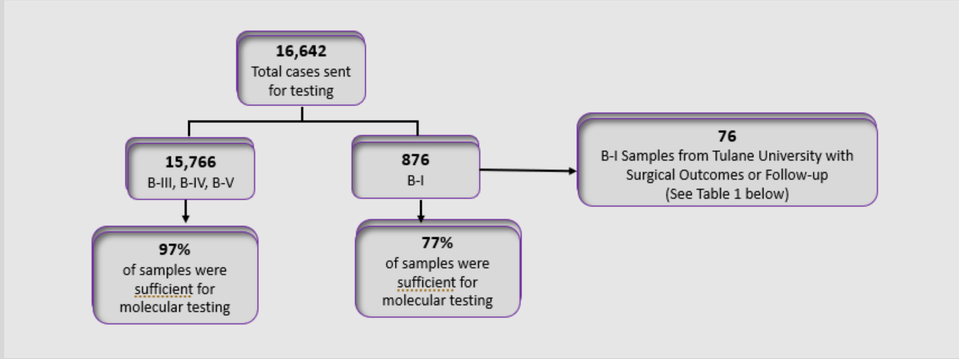
1. Examine the diagnostic value of a combination approach using a mutation panel and a microRNA classifier in B-I samples.
2. Examine the diagnostic value of a combination molecular test platform (mutational panel + miRNA classifier) in a cohort of B-I samples with known clinical outcomes or follow-up.

Methods

- Interpace reviewed the molecular test results of 16,642 consecutive thyroid FNAs submitted for NGS-mutation testing (ThyGenX®/ThyGeNEXT®) in combination with miRNA expression classification (ThyraMIR®)
- This set included 876 B-I cases and 15,766 cases with a B-III, B-IV, or B-V cytology diagnosis which were collected from numerous centers
- Samples were deemed to be sufficient for testing based upon established laboratory standards for nucleic acid quality and follicular cell origin in a CAP accredited and CLIA certified laboratory
- Within this cohort was a set of 76 B-I samples from Tulane University for which the molecular test results were compared to the real-world clinical outcomes and/or clinical follow-up
- It is assumed that the data from the additional 800 cases, from multiple centers, should provide similar results

Results

Figure 1: Total Cohort: 16,642 samples



- 77% and 97% of FNAs with B-I vs B-III, B-IV, and B-V cytology samples were sufficient for molecular testing by NGS, respectively; 78% and 97% of samples with B-I vs B-III, B-IV, and B-V cytology were sufficient for ThyraMIR® testing, respectively
- Although the sample sufficiency rate of B-I cytology samples was different than those for B-III, B-IV, and B-V cytology, the quality of the molecular testing data for the sufficient samples was similar for all cytology categories

Table 1: Tulane University Cohort (n=76)

Sample #	Gender	Age	Size of Nodule	Location	Mutation Panel Result	ThyraMIR® Result
1	M	62	5	LT MID	Negative	Negative
2	M	46	1.2	RT UPPER	Negative	Negative
3	F	70	3.81	RT	Negative	Negative
4	F	53	NA	LT	Negative	Negative
5	F	60	1.1	LT LOW	Negative	Negative
6	F	57	1.18	RT MID	Negative	Negative
7	F	54	1.7	RT MID	Negative	Negative
8	F	78	0.9	RT MID	Negative	Negative
9	F	57	1.3	LT MID UP	Negative	Positive
10	M	62	1.1	RT LOW	Negative	Negative
11	F	21	3.2	LT MID	NRAS Q61K	Negative
12	F	75	0.94	LT UPPER	Insufficient	Insufficient
13	F	79	NA	RT	Negative	Negative
14	F	63	1.12	RT LOW	Negative	Negative
15	F	42	0.73	RT	Negative	Negative
16	M	57	1.25	RT LOW	Negative	Negative
17	F	66	1.2	LT MID	Negative	Negative
18	F	70	1.83	LT UPPER	Negative	Negative
19	F	56	0.84	ISTHMUS	Negative	Negative
20	F	71	1.5	RT LOW	Negative	Negative
21	F	60	1.9	RT LOW	Negative	Negative
22	F	61	0.9	RT MID	Negative	Negative
23	F	58	NA	RT MID	Negative	Negative
24	F	49	2.16	LT UPPER	Negative	Negative
25	F	67	0.9	RT UPPER	Negative	Negative
26	F	80	2.29	RT MID	Negative	Negative
27	F	89	1.62	RT LOWER	Negative	Negative
28	M	63	2.36	LT UPPER	Negative	Negative
29	F	72	0.78	RT MID	Negative	Negative
30	F	44	1.38	RT UPPER	Insufficient	Insufficient
31	F	78	0.7	LT LOW	Insufficient	Insufficient
32	F	44	NA	LT MID	Insufficient	Insufficient
33	F	49	1.59	RT MID	Insufficient	Insufficient
34	F	49	1.4	LT LOWER	Insufficient	Insufficient
35	F	21	1.41	RT LOW	Insufficient	Insufficient
36	F	50	1.09	LT CTR	Insufficient	Insufficient
37	M	63	1.6	LT LOW	Negative	Negative
38	M	63	1.4	LT MID	Insufficient	Insufficient
39	M	80	1.2	RT LOW	Negative	Negative
40	M	69	2.2	RT UPP	Negative	Negative
41	F	60	3.2	RT LOW	Insufficient	Insufficient
42	F	82	NA	LT MID	Negative	Negative
43	F	82	NA	LT LOW	Negative	Negative
44	F	82	1.2	RT LOW	Negative	Negative
45	F	52	1.76	ISTHMUS	Negative	Negative
46	F	50	1	RT MID	Negative	Negative
47	F	60	2.68	LT MID	Insufficient	Insufficient
48	F	64	NA	LT MID	Insufficient	Insufficient
49	F	64	0.75	LT CTR	Negative	Negative
50	F	59	1.4	LT MID	Negative	Negative
51	F	77	1.2	RT MID	Negative	Negative
52	F	36	1.2	RT LOW	Insufficient	Insufficient
53	F	70	1.23	RT MID	Negative	Negative
54	F	56	0.9	LT	Negative	Insufficient
55	F	78	1	LT LOW	Insufficient	Insufficient
56	F	58	1	RT LOW	Negative	Negative
57	F	85	2.46	LT MID	Negative	Negative
58	F	47	2.59	LT LOW	Negative	Negative
59	F	49	2.3	ISTH	Negative	Negative
60	F	45	NA	LT LOW	Negative	Negative
61	F	52	1.46	RT MID	Negative	Negative
62	F	26	1.9	RT	Negative	Negative
63	F	69	1.1	RT LOW	Negative	Insufficient
64	M	58	1.9	LT MID	Insufficient	Insufficient
65	F	28	2.54	ISTHMUS	GNAS (Q227H)	Negative
66	F	70	1	LT LOW	Insufficient	Insufficient
67	F	70	1	RT LOW	Negative	Insufficient
68	F	59	2.7	RT LOW	Negative	Negative
69	F	59	2.9	LT LOW	Negaitve	Negative
70	F	67	1.6	LT	Negative	Negative
71	F	55	1.2	LT	Negative	Negative
72	F	46	1.45	RT MID	Negative	Negative
73	M	58	1.8	RT	Negative	Negative
74	F	72	4.4	LT LOW	Negative	Negative
75	F	51	0.5	RT	NRAS Q61K	Negative
76	M	33	5	LT	Negative	Negative

- 76 B-I samples were collected between February 2018 and April 2019 and sent by Tulane University to Interpace Diagnostics for testing with ThyGenX®/ThyGeNEXT® and ThyraMIR® (Tulane University Biomedical IRB, Ref. # 2019-1081)
- 79% of samples tested (60/76) received a successful molecular result for both the mutation panel and the miRNA classifier
- 80% (61/76) received a successful result for the mutation panel

Results

Table 2: Tulane B-I Cases with Surgical Outcomes

Sample #	Gender	Age	Size of Nodule	Location	Mutation Panel Result	ThyraMIR® Result	Surgical Pathology
1	M	62	5	LT MID	Negative	Negative	Nodular Hyperplasia
2	M	46	1.2	RT UPPER	Negative	Negative	Nodular Hyperplasia
3	F	70	3.81	RT	Negative	Negative	Nodular Hyperplasia
4	F	53	NA	LT	Negative	Negative	Follicular Adenoma
5	F	60	1.1	LT LOW	Negative	Negative	Nodular Hyperplasia
6	F	57	1.18	RT MID	Negative	Negative	Nodular Hyperplasia
7	F	54	1.7	RT MID	Negative	Negative	Benign
8	F	78	0.9	RT MID	Negative	Negative	Micro PTC (1mm)
9	F	57	1.3	LT MID UPPER	Negative	Positive	Nodular Hyperplasia
10	M	62	1.1	RT LOW	Negative	Negative	Follicular Adenoma
11	F	21	3.2	LT MID	NRAS Q61K	Negative	fv PTC
12	F	75	0.94	LT UPPER	Insufficient	Insufficient	Micro PTC (1mm)

- 12 patients with B-I cytology underwent surgical intervention, and one had insufficient material for molecular testing
- The molecular test results matched the surgical pathological diagnosis in 10 of the 11 patients who had samples sufficient for molecular testing

Conclusions

- A combined molecular testing approach utilizing a mutation panel along with a miRNA classifier can provide useful diagnostic information for cases where the cytology results for thyroid FNAs were classified as B-I
- Use of molecular testing with ThyGeNEXT® and ThyraMIR® can reduce the need for repeat FNA procedures in many cases, saving healthcare costs and improving patient quality of life

Acknowledgements and Works Cited

1. Alexander EK et al. *J Clin Endocrinol Metab*. 2002;87(11):4924-4927
2. Abstract – 2016 ATA Meeting. The Majority of Non-Diagnostic (Insufficient) Thyroid Nodule Cytology Samples Can Effectively Udergo Molecular (Combined Mutational and MicroRNA Classifier) Analysis Using a Needle Aspiration Approach.