Clinical Utility of a Mutation Panel Used in Combination With an Expanded microRNA Risk Classifier in Indeterminate Thyroid Nodules: A Large Real-world Observational Study

Tanvi Verma MD[1]; Joseph Del Tondo DO[1]; Cody Marshall DO[1]; Kossivi Dantey MD[1];
Diane V. Thompson MS[2]; Anna Banizs MD[3]; Sydney Finkelstein MD[3]

1. Allegheny Health Network, Allegheny, PA. 2. Diane Vido Thompson, LLC, Murrysville, PA. 3.Interpace Diagnostics, Parsippany, NJ.

2022 Annual Meeting
MONTRÉAL, QUEBEC, CANADA
OCTOBER 19 – 23, 2022

Highlighted Poster #119

BACKGROUND

Molecular analysis of fine-needle aspiration biopsies (FNAB) improves the diagnostic accuracy of indeterminate thyroid nodules (ITNs) as previously demonstrated with the use of multiplatform molecular testing (MPTX) combining mutational analysis (ThyGeNEXT®) with quantitative microRNA (miRNA) expression (ThyraMIR®).[1] Recently, MPTXv2 (ThyGeNEXT + ThyraMIR®v2) has been shown to further improve the accurate risk stratification of ITNs with the use of miRNA pairwise expression.[2]

OBJECTIVE

Here we seek to assess the clinical utility of MPTX (ThyGeNEXT® + ThyraMIR®) in a prospectively collected, consecutive, real-world clinical cohort. We also aim to perform a secondary, retrospective analysis, using a novel Benign/Malignant miRNA pairwise expression profiler (ThyarMIR®v2) in conjunction with ThyGeNEXT® (MPTXv2).

METHODS

338 patient samples with a B-III (n=260) or B-IV (n=78) cytology diagnosis and with corresponding surgical outcomes or long-term follow-up, collected at 3 different centers within the Allegheny Health Network between 2016 and 2020 were included. All samples underwent multiplatform testing (MPTX), which includes an oncogene panel (ThyGeNEXT®) plus a microRNA Risk Classifier (ThyraMIR®). More recently, a secondary blinded molecular analysis was performed to assess the added utility of MPTXv2. Test results are reported as either Negative (low-risk), Positive (high-risk), or Moderate-Risk. The average length of clinical follow-up/surveillance was 30 months (n=247). The cohort consisted of 293 negative/currently negative (low-risk) samples and 45 positive (malignant) samples; the rate of disease for this cohort is 14%.

RESULTS

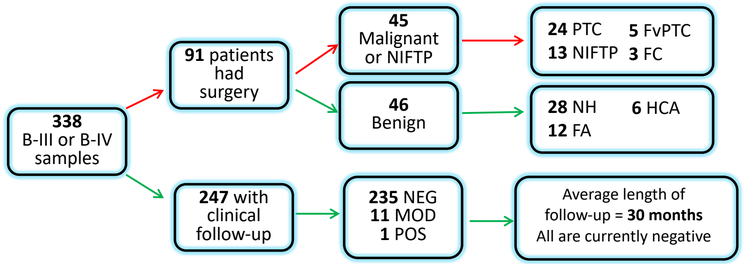

Figure 1. Distribution of Samples

PTC=Papillary Thyroid Cancer; **FvPTC**=Follicular Variant of Papillary Thyroid Cancer; **NIFTP**=non-invasive follicular thyroid neoplasm with papillary-like nuclear features; **FC**=Follicular Cancer; **NH**=Nodular Hyperplasia; **HCA**=Hürthle Cell Adenoma; **FA**=Follicular Adenoma.

RESULTS

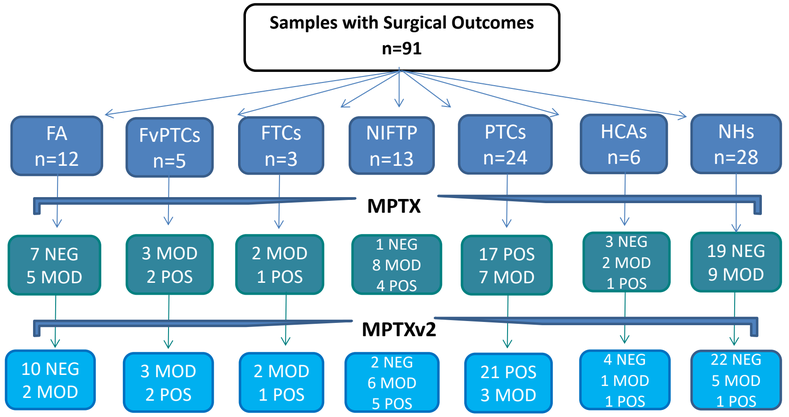

Figure 2. MPTX and MPTXv2 test results (negative, moderate-risk, or positive, for samples with surgical outcomes. The use of MPTXv2 reduced the number of Moderate-Risk samples by 12 for this surgical cohort and the number of Moderate-Risk samples were reduced by 22 for the entire cohort of 338.

Cytology Dx	Mutation Type	ThyGeNEXT®	ThyraMIR®	MPTX	MPTXv2	Diagnosis	Histopath Dx
B-III	*BRAF*-like	*STRN/ALK*	Level-3	POS	POS	Malignant	PTC
B-III	*BRAF*-like	*BRAF* V600E	Level-3	POS	POS	Malignant	PTC
B-III	*BRAF*-like	*BRAF* V600E	Level-3	POS	POS	Malignant	PTC
B-III	*BRAF*-like	*HRAS* + *TERT*	Level-3	POS	POS	Malignant	PTC
B-III	*BRAF*-like	*NRAS* + *TERT*	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*BRAF* V600E	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*BRAF* V600E	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*BRAF* V600E	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*BRAF* V600E	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*BRAF* V600E	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*BRAF* V600E + *TERT*	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*BRAF* V600E + *TERT*	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*NRAS* + *TERT*	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*TERT* + *NRAS*	Level-3	POS	POS	Malignant	PTC
B-IV	*BRAF*-like	*TERT* + *NRAS*	Level-3	POS	POS	Malignant	PTC

Table 1. Summary of samples containing a *BRAF* V600E-like mutation (n=15).

- 7 samples contained an isolated *BRAF* V600E mutation
- 2 samples contained both a *BRAF* V600E and a *TERT* promoter mutation
- 1 samples contained a *TERT* promoter mutation along with an HRAS mutation
- 4 samples contained a *TERT* promoter mutation along with an NRAS mutation
- 1 sample contained a *STRN/ALK* fusion
- All 15 cases with identified *BRAF* V600E-like mutations were diagnosed correctly by both MPTX and MPTXv2 and malignancy was confirmed by surgical histology

RESULTS

- 235/338 samples received negative results (low risk of malignancy) allowing these patients to avoid an unnecessary surgery and potential associated morbidity
- 70% Benign Call Rate (BCR). A high BCR has been associated with reduced healthcare costs.[3]
- Some patient with negative results elected to have surgery primarily due to compressive symptoms, nodule growth or cosmetic reasons
- All 247 patients who have undergone standard clinical follow-up (repeat US every 6-12 months) remain currently benign, with an average length of clinical follow-up of 30 months (actual range of 8-72 months)
- There were no false negatives among the patients with negative test results with long-term follow-up (Figure 1)
- MPTXv2 provided improved risk stratification of ITN vs. MPTX alone
- The mutation panel identified 30 *RAS*-like mutations in the benign cohort and 20 *RAS*-like mutations in the malignant cohort
- The Moderate-Risk group was reduced from 55 samples with MPTX to 33 samples using MPTXv2
- 21/22 Moderate-Risk samples, with surgical outcomes, were correctly reclassified as benign or malignant with MPTXv2. There was 1 NIFTP sample that was incorrectly classified as NEG by MPTXv2
- The sensitivity was 94% for MPTX and 96% for MPTXv2. The specificity was 99% for both MPTX and MPTXv2 and the NPV at negative threshold was 99% for both MPTX and MPTXv2. At 14% ROD the PPV at positive threshold was 92% and 89% for MPTX vs MPTXv2. At a 30% Disease Prevalence, the NPV was 97% for MPTX vs 98% for MPTXv2 and the PPV was 95% for MPTX vs 97% for MPTXv2

CONCLUSIONS

Our data suggest that both MPTX and MPTXv2 accurately risk-stratified ITNs. Blinded analysis of pairwise miRNA expression profiling (MPTXv2) further improves the accuracy of MPTX, resulting in a reduction in the number of samples in the Moderate-Risk group and a decrease in the false-positive rate, thereby allowing more patients to avoid potentially unnecessary surgery. Use of MPTXv2 resulted in a high BCR of 70%, allowing more patients to avoid unnecessary surgeries.

REFERENCES

1. Lupo MA, Walts AE, Sistrunk JW, et al. Multiplatform molecular test performance In indeterminate thyroid nodules. *Diagn Cytopathol*. 2020;48:1254–1264.

2. Finkelstein SD, et al. A retrospective evaluation of the diagnostic performance of an interdependent pairwise microRNA expression analysis with a mutation panel in indeterminate thyroid nodules [published online ahead of print, August 9, 2022]. *Thyroid*. doi:10.1089/thy.2022.0124.

3. Labourier E. Utility and cost effectiveness of molecular testing in thyroid nodules with indeterminate cytology. *Clin Endocrinol*. 2016;3-8. doi.10.1111/cen.13096.