Prospective Evaluation of ThyroSPEC Molecular Testing of Indeterminate Thyroid Nodule Cytologies Following Diagnostic Pathway Optimization

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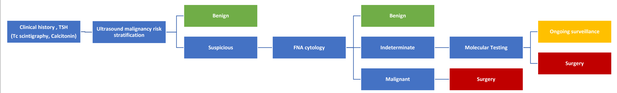
Introduction

- Thyroid nodules are highly prevalent and typically identified incidentally or from symptoms or palpation, which necessitates a clinical evaluation and ultrasound to assess malignancy risk
- Quality ultrasound malignancy risk assessment allows rule-out of malignancy in ~50% of thyroid nodules, but historical local reporting was inadequate[1] to risk stratify nodules according to guidelines
- Suspicious nodules on ultrasound are referred to thyroid nodule fine needle aspiration cytology (FNAC), where malignancy is ruled out in another 60% of nodules. However, up to 30% of FNACs are indeterminate due to an inherent limitation of cytology with diagnostic surgery as the standard of care
- Following evaluation of malignancy and resection rates across 5867 thyroid FNAC in Calgary[2], implementation of guidelines-based ultrasound reporting[1], and implementation of a local thyroid nodule diagnostic pathway with the Calgary PCNs[3], we implemented reflexive ThyroSPEC™ testing in Calgary for indeterminate thyroid FNAC molecular diagnosis
- In this ongoing study we examine the outcomes of implementing the above improvements in the local diagnostic pathway



ThyroSPEC™

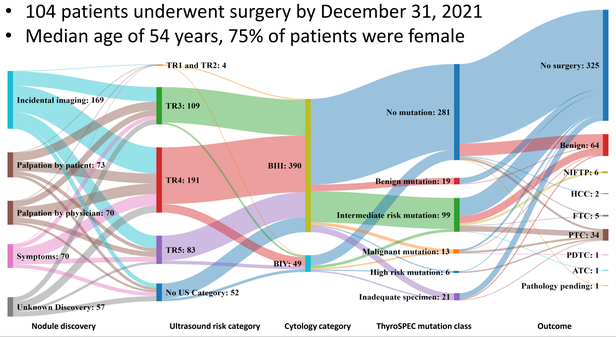
The ThyroSPEC™ panel covers the most prevalent **140** point mutations and gene fusions in thyroid cancer, including all thyroid carcinoma mutations described more than once in the COSMIC database v83:

117 point mutations in			23 rearrangements involving		Gene Expression of	
AKT1	EZH1	RET	ALK	PPARG	CALCA	PGK1
BRAF	HRAS	SPOP	BRAF	PRKAB1	KRT7	PTH
CTNNB1	IDH1	TERT	MAML2	RET	KRT20	TG
DICER1	KRAS	TP53	NTRK1	THADA	PAX8	TTF1
EGFR	NRAS	TSHR	NTRK3			
EIF1AX	PIK3CA					

- ThyroSPEC™ inputs include DNA and RNA extracted from residual liquid FNAC material left over after preparation of slides
- Highly multiplexed genotyping on the MassARRAY MS platform (Agena Biosciences) is faster and at lower cost per sample than NGS
- Diagnosis by ThyroSPEC™ is graded according to the risk of malignancy of the specific mutation(s)[3]

Cohort

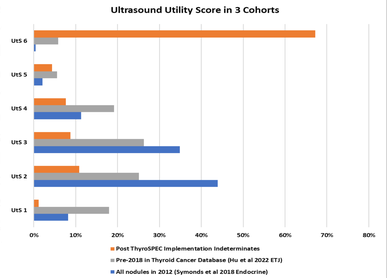
- 439 consecutive patients with indeterminate thyroid FNAC collected from July 29, 2020, until December 31, 2021
- 89% AUS/FLUS (Bethesda III), 11% FN/SFN (Bethesda IV) on cytology
- 104 patients underwent surgery by December 31, 2021
- Median age of 54 years, 75% of patients were female



Optimized Pathway

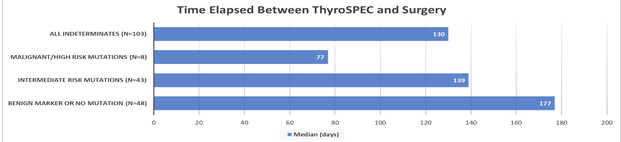
Following collaboration with local radiology practices, guidelines-based thyroid nodule ultrasound malignancy risk stratification was recently implemented locally[1].

The ultrasound utility score (report quality measured by the number of documented nodule features for each ultrasound) significantly increased ($p<0.01$) from historical levels[4], this means that guidelines-based ultrasound malignancy risk stratification is now available for clinical decision making alongside ThyroSPEC™ for nearly all indeterminate FNAC.



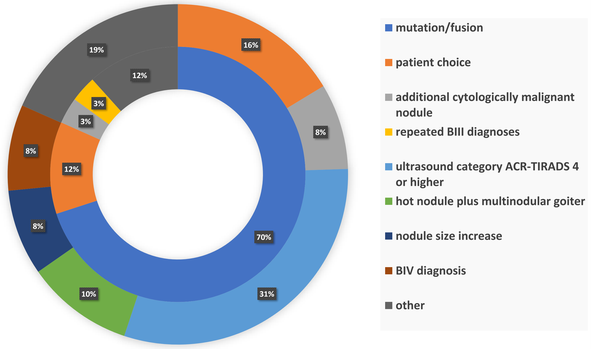
Improved Surgical Triage

Malignant mutations expedite surgery by 100 days ($p<0.01$) compared to indeterminates without mutations, thus ThyroSPEC™ enables rational prioritization.



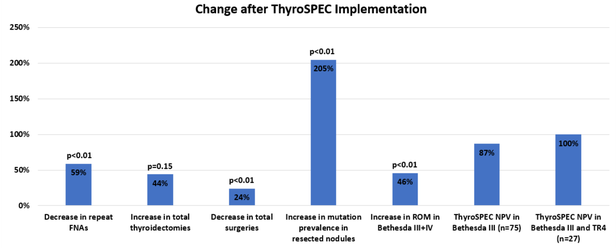
Results

Primary reason for surgery in ThyroSPEC™ positive (inner ring) and ThyroSPEC™ negative (outer ring) patients with a resected, cytologically indeterminate thyroid nodule



Thus, since implementation of reflex ThyroSPEC™ testing, mutation status is the predominant reason for surgery in ThyroSPEC™ positive resected nodules which demonstrates a direct impact of ThyroSPEC™ on clinical decision-making.

The following graph shows the differences in outcomes before and after ThyroSPEC™ implementation, as well as the NPV of ThyroSPEC™.



Since implementation of reflex ThyroSPEC™ testing and optimization of the upstream components of the local thyroid nodule diagnostic pathway, unnecessary diagnostic procedures including FNAs and surgeries have been avoided while diagnostic yield has increased. Molecular diagnostics has provided accurate, incremental pre-surgical malignancy risk stratification, thereby improving referral patterns and surgical triage which directly benefits patients.

Conclusions

Molecular data in the context of an optimized diagnostic pathway provides the following clinical benefits:

- **Improved surgical triage for FNAC with high risk mutations and a far higher rate of surgery in ThyroSPEC™ positive than negative patients**
- **Genetic alterations identified by ThyroSPEC™ are the predominant reason for surgery in ThyroSPEC™ positive indeterminate FNAC, demonstrating direct impact of molecular diagnostics on clinical decision-making**
- **Reduced repeat FNAs, completion thyroidectomies, and total surgeries provide immediate reductions in patient morbidity resulting from overtreatment and represent cost savings achieved through a locally optimized thyroid nodule diagnostic pathway**
- **Despite fewer overall surgeries, more malignant nodules have been resected since ThyroSPEC™ implementation through a significantly improved diagnostic yield compared to historical cohorts**

Next Steps

- This study is ongoing to generate sufficient power for the analysis of subsets such as ThyroSPEC™ performance in intermediate suspicion ultrasound categories where decision making is most differential
- We are working on multiple projects to improve the sensitivity of ThyroSPEC™ with the goal to improve sensitivity enough for ThyroSPEC™ to become a rule-out test, such that a negative (wild-type) result could rule-out malignancy
- We intend to create an integrated, clinically applicable regression classifier that combines ultrasound, cytology, and molecular data to estimate a personalized risk of malignancy for each patient and provide a locally validated treatment algorithm
- For more information, reach out to: contact@thyrospec.com

Abbreviations

AUS/FLUS or Bethesda III (BIII), atypia of undetermined significance / follicular lesion of undetermined significance; FN/SFN or Bethesda IV (BIV), follicular neoplasm / suspicious for a follicular neoplasm; FNAC, fine needle aspiration cytology; MS, mass spectrometry; NGS, next generation sequencing; NPV, negative predictive value; ROM, risk of malignancy.

References

1. Hu, X.Y., Wu, J., Seal, P., Ghaznavi, S., Symonds, C.J., Kinnear, S.E. and Paschke, R., 2022. Improvement in thyroid ultrasound report quality with radiologists' adherence to 2015 ATA or 2017 TIRADS: a population study. *European Thyroid Journal*, *1*(aop).
2. Ghaznavi, S.A., Clayton, H., Eszlinger, M., Khalil, M., Symonds, C.J. and Paschke, R., 2022. Accuracy of Thyroid Fine-Needle Aspiration Cytology: A Cyto-Histologic Correlation Study in an Integrated Canadian Health Care Region with Centralized Pathology Service. *Acta Cytologica*, *66*(3), pp.171-178.
3. ThyroSPEC™ recommendations and the Calgary PCN diagnostic pathway: bit.ly/thyrospec, bit.ly/3ycCZxP
4. Symonds, C.J., Seal, P., Ghaznavi, S., Cheung, W.Y. and Paschke, R., 2018. Thyroid nodule ultrasound reports in routine clinical practice provide insufficient information to estimate risk of malignancy. *Endocrine*, *61*(2), pp.303-307.

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