A novel and promising proteomic-based MALDI-MSI thyroid nodule classifier as complementary diagnostic tool in cytopathology

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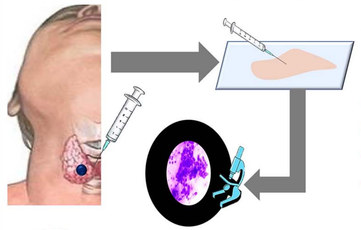
INTRODUCTION

THYROID FINE NEEDLE ASPIRATION (FNA) BIOPSY

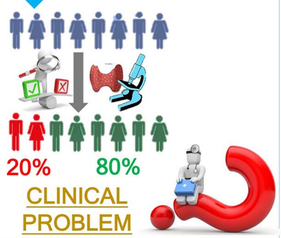


SIAPEC-IAP Classification

THY1: Inadequate sampling
THY2: Benign lesions
THY3: Indeterminate diagnosis
THY4: Suspicious for malignancy
THY5: Malignant lesions

TOTAL THYROIDECTOMY
25% FNA (THY3)

- Public health cost
- Surgical stress/complications
- Lifelong hormone therapy



AIMS

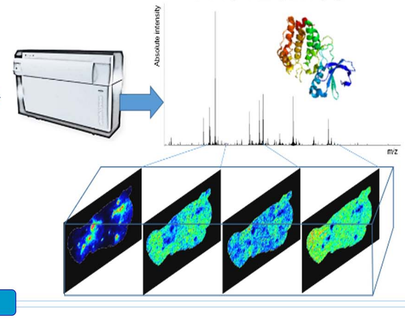
- Discriminate Benign and Malignant thyroid nodules
- Classify Indeterminate (THY3) nodules.

SAMPLE PREPARATION METHOD

- Reproducible and Robust
- Specific and sensitive
- Transferable in different clinics

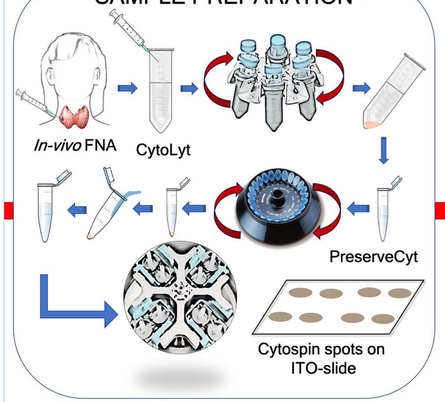
In-vivo FNA

MALDI-MSI PROTEOMICS

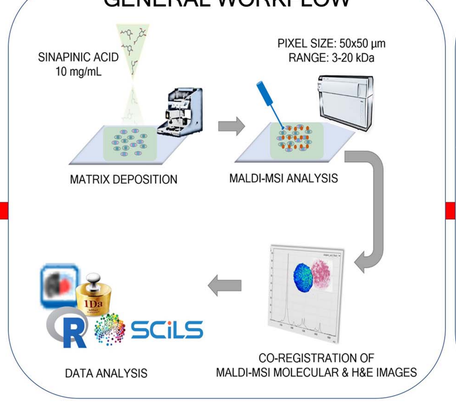


METHODS

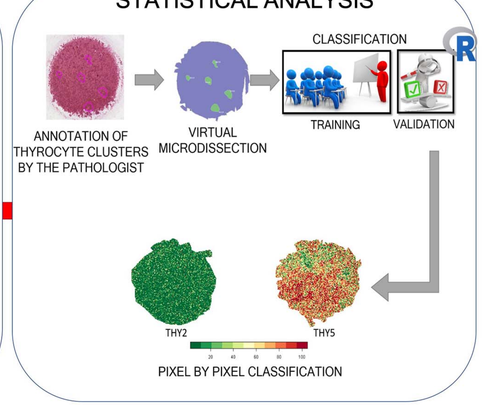
SAMPLE PREPARATION[1,2]



GENERAL WORKFLOW



STATISTICAL ANALYSIS



RESULTS

TRAINING: ROI
N = 9 hyperlplastic patients (THY2)
N = 9 Papillary Thyroid Carcinoma patients (THY5)

Equivalent group of ROIs were generated for each patient:
- 5 groups of ROIs for THY2 (Total: 45 ROIs)
- 4 groups of ROIs for THY5 (Total: 36 ROIs)

20 discriminant features

LASSO MODEL

VALIDATION
n=4 THY2; n = 1 THY3; n=1 THY4, n=4 THY5; n=1 metastatic lymph node

- Overall Average Spectrum
- Regions of Interest Spectra
- Single Spectrum: Pixel by Pixel

PREPROCESSING PARAMETERS
- TIC Normalization
- S/N ≥ 6 and intensity threshold ≥ 0.0003
- Intra-Patient Filter (Only features detected in at least 25% of the ROIs)
- Inter-Patient Filter (Only features common to at least 25% of the Thy2 or to 25% of the Thy5)

Including criteria: Only ROI with at least 3 spectra

Pixel by pixel Images & Histograms of Frequency[3]

BENIGN

TRAINING SAMPLE

P_308 (THY2)

VALIDATION SAMPLES

P_1081 (THY2)
P_1083 (THY2)
P_1123 (THY2)
P_1156 (THY2)

MALIGNANT

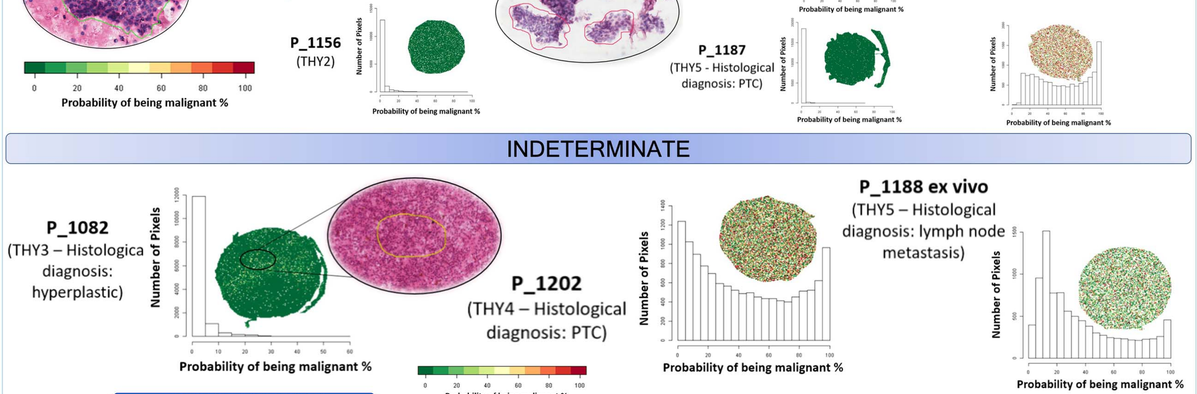
TRAINING SAMPLE

P_250 (THY5)

VALIDATION SAMPLES

P_1084 (THY5 - Histological diagnosis: PTC)
P_1126 (THY5 - Histological diagnosis: PTC)
P_1149 (THY5 - Histological diagnosis: PTC)
P_1187 (THY5 - Histological diagnosis: PTC)

IN-VIVO
EX-VIVO

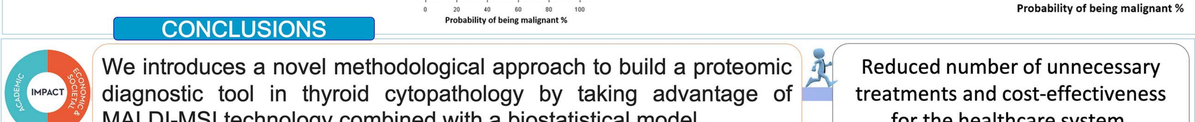


INDETERMINATE

P_1082 (THY3 – Histologica diagnosis: hyperplastic)

P_1202 (THY4 – Histological diagnosis: PTC)

P_1188 ex vivo (THY5 – Histological diagnosis: lymph node metastasis)



CONCLUSIONS

We introduces a novel methodological approach to build a proteomic diagnostic tool in thyroid cytopathology by taking advantage of MALDI-MSI technology combined with a biostatistical model.

Reduced number of unnecessary treatments and cost-effectiveness for the healthcare system.

REFERENCES:
[1] Piga I et al., The management of haemoglobin interference for the MALDI-MSI proteomics analysis of thyroid fine needle aspiration biopsies. Analytical and Bioanalytical Chemistry. 411, pages 5007-5012 (2019).
[2] Piga I et al., Feasibility Study for the MALDI-MSI Analysis of Thyroid Fine Needle Aspiration Biopsies: Evaluating the Morphological and Proteomic Stability Over Time. Proteomics Clinical Application. 13(1):e1700170 (2019).
[3] Capitoli G et al., MALDI-MSI as a Complementary Diagnostic Tool in Cytopathology: A Pilot Study for the Characterization of Thyroid Nodules.Cancers. 11(9), 1377 (2019).

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