Combination of serum miRNA-190 and -95 is a powerful non-invasive tool in the differential diagnosis of thyroid nodules: preliminary data of a prospective series.

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Introduction

MicroRNAs (miRNAs) are small non-protein encoding RNAs which negatively regulate gene expression.
Tissue miRNA profiles may be useful to distinguish benign from malignant lesions.
Recently, we have identified in the serum of a retrospective series of patients, with benign nodular goiter (n=80) and papillary thyroid cancer (PTC: n=79), 2 miRNAs (-190 and -95) that in combination allow the differential diagnosis of thyroid nodules with great accuracy.

Aim

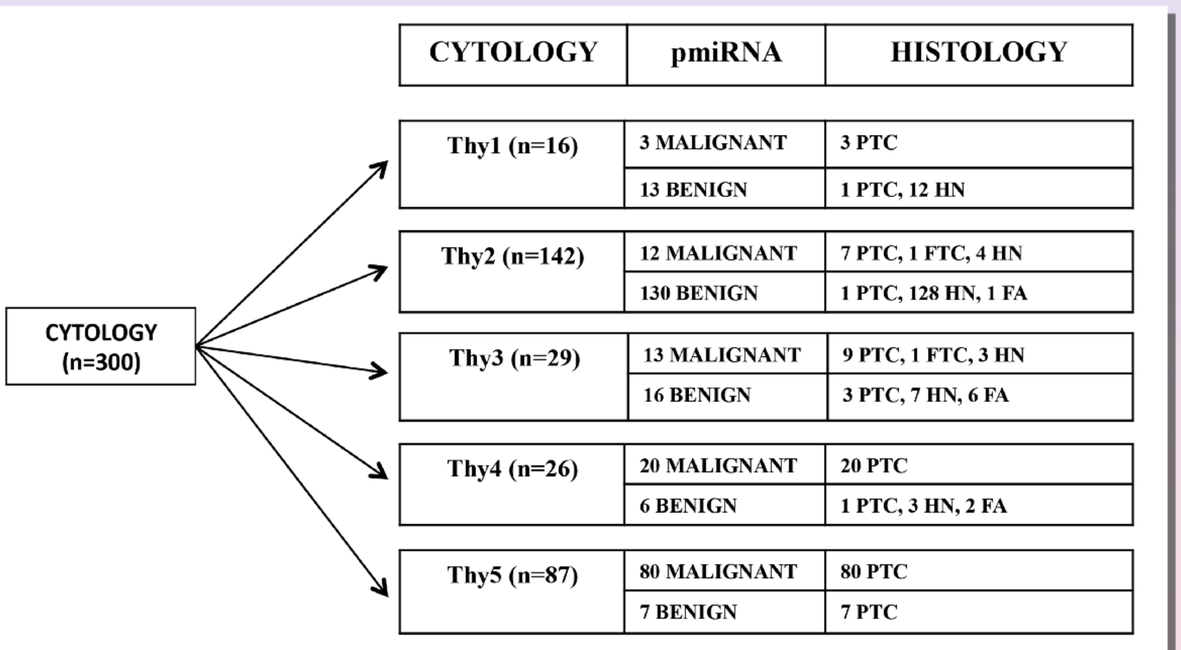
This study was aimed to confirm the diagnostic accuracy of miRNA-190 and -95 in a prospective series.

Patients and Methods

- ✓ A total of 473 patients have been enrolled in our study (159 patients retrospectively and 314 prospectively). All patients underwent fine needle aspiration cytology at our Institute and 300 out of 473 patients were treated surgically and the histology is available.
- ✓ MiRNAs were extracted from serum using the miRNeasy Serum/Plasma kit (Qiagen), and reverse-transcribed using Megaplex Human microRNA RT primers pools v2.1 (Life Technologies). Relative expression quantification was evaluated by the comparative cycle threshold (CT) method ($2^{-\Delta\Delta Ct}$) (Rotor-gene Q, Qiagen).
- ✓ We developed a mathematical formula which calculates the probability of malignancy (pmiRNA) with a cut-off value of 0.5 above which the patient was at high risk of malignancy.

Results

Cytological Series with histology

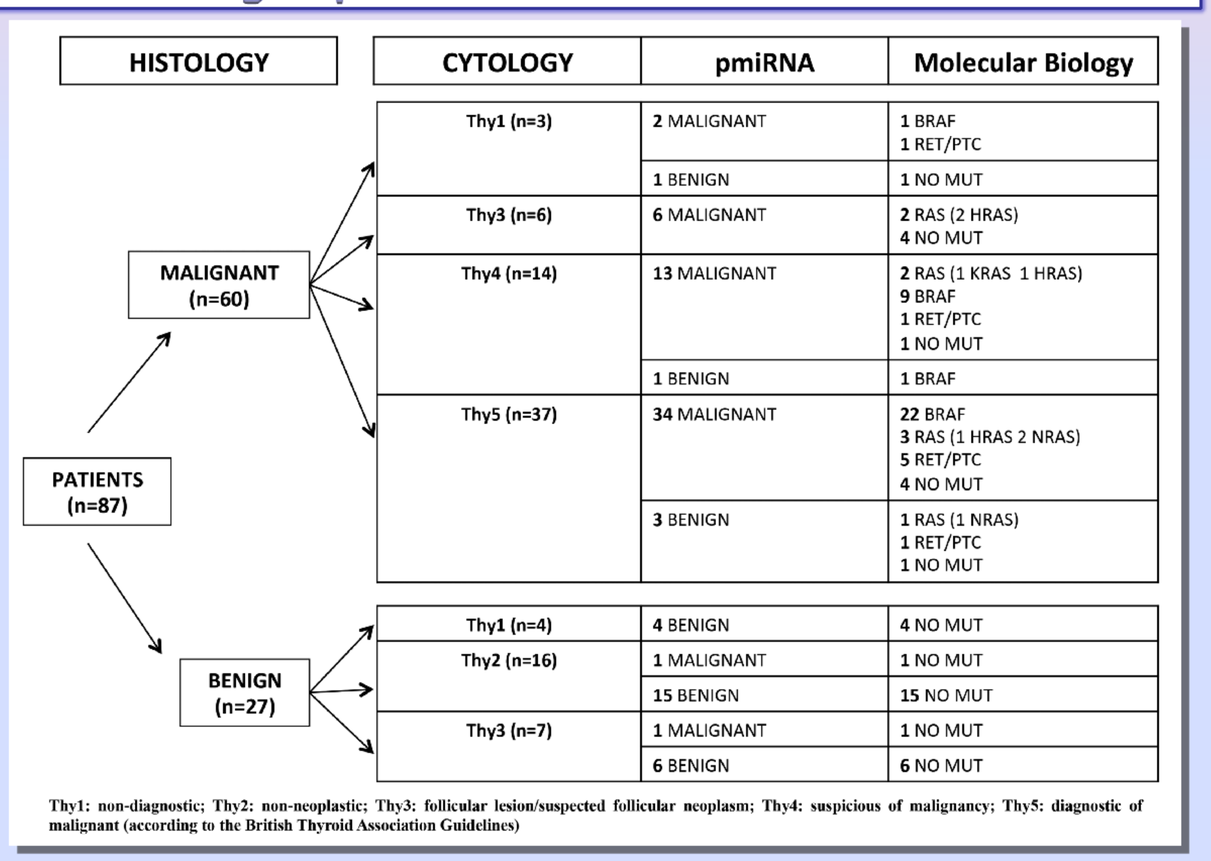


CYTOLOGY	pmiRNA	HISTOLOGY
Thy1 (n=16)	3 MALIGNANT	3 PTC
	13 BENIGN	1 PTC, 12 HN
Thy2 (n=142)	12 MALIGNANT	7 PTC, 1 FTC, 4 HN
	130 BENIGN	1 PTC, 128 HN, 1 FA
Thy3 (n=29)	13 MALIGNANT	9 PTC, 1 FTC, 3 HN
	16 BENIGN	3 PTC, 7 HN, 6 FA
Thy4 (n=26)	20 MALIGNANT	20 PTC
	6 BENIGN	1 PTC, 3 HN, 2 FA
Thy5 (n=87)	80 MALIGNANT	80 PTC
	7 BENIGN	7 PTC

Thy1: non-diagnostic; Thy2: non-neoplastic; Thy3: follicular lesion/suspected follicular neoplasm; Thy4: suspicious of malignancy; Thy5: diagnostic of malignant (according to the British Thyroid Association Guidelines)
PTC papillary thyroid cancer; FTC: follicular thyroid cancer; HN: hyperplastic nodule; FA: follicular adenoma

	FNAC	pmiRNA	FNAC+pmiRNA
Sensitivity (%)	92.3	92.3	99.1
Specificity (%)	96.3	93.6	93.5
Positive Predictive Value (%)	92.3	96.4	92.8
Negative Predictive Value (%)	93.6	93.7	99.2
Diagnostic accuracy (%)	94.5	94.9	96

Subgroup with known mutational status



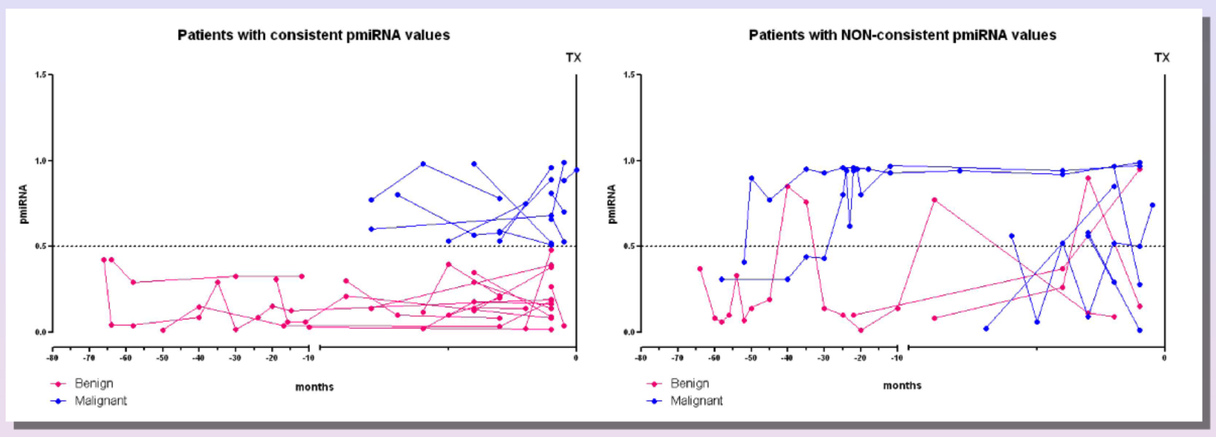
HISTOLOGY	CYTOLOGY	pmiRNA	Molecular Biology
MALIGNANT (n=60)	Thy1 (n=3)	2 MALIGNANT	1 BRAF 1 RET/PTC
		1 BENIGN	1 NO MUT
	Thy3 (n=6)	6 MALIGNANT	2 RAS (2 HRAS) 4 NO MUT
	Thy4 (n=14)	13 MALIGNANT	2 RAS (1 KRAS 1 HRAS) 9 BRAF 1 RET/PTC 1 NO MUT
		1 BENIGN	1 BRAF
	Thy5 (n=37)	34 MALIGNANT	22 BRAF 3 RAS (1 HRAS 2 NRAS) 5 RET/PTC 4 NO MUT
		3 BENIGN	1 RAS (1 NRAS) 1 RET/PTC 1 NO MUT
BENIGN (n=27)	Thy1 (n=4)	4 BENIGN	4 NO MUT
	Thy2 (n=16)	1 MALIGNANT	1 NO MUT
		15 BENIGN	15 NO MUT
	Thy3 (n=7)	1 MALIGNANT	1 NO MUT
		6 BENIGN	6 NO MUT

Thy1: non-diagnostic; Thy2: non-neoplastic; Thy3: follicular lesion/suspected follicular neoplasm; Thy4: suspicious of malignancy; Thy5: diagnostic of malignant (according to the British Thyroid Association Guidelines)

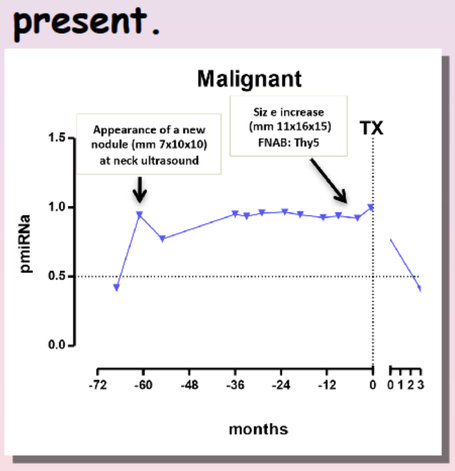
	# of cases correctly identified (%)		
	pmiRNA	molecular biology (mol biol)	pmiRNA+mol biol
THY1	6/7 (85.7)	6/7 (85.7)	6/7 (85.7)
THY2	15/16 (93.7)	16/16 (100)	15/16 (93.7)
THY3	12/13 (92.3)	9/13 (69.2)	12/13 (92.3)
THY4	13/14 (92.8)	13/14 (92.8)	14/14 (100)
THY5	34/37 (91.9)	32/37 (86.5)	36/37 (97.3)

MicroRNA expression levels over the time

- ✓ We calculated pmiRNA value in different serum samples (# 2-17 for each patients) of 36 patients (17 patients with thyroid cancer and 19 with benign nodules) over 65 months before surgery. In 26/36 patients pmiRNA value was consistent in all determinations for each patient.



- ✓ In one case with discrepant findings at the first sample the cancer might not be present.



Conclusions

- ✓ PmiRNA showed a high sensitivity (92.3%), specificity(93.6%) and diagnostic accuracy (94.9%) in PTC diagnosis.
- ✓ These preliminary data confirm that pmiRNA may be useful as non-invasive diagnostic tool for the differential diagnosis of thyroid nodules, particularly in case of indeterminate lesions where pmiRNA role is promising but further studies are warranted.

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