Thyroid Nodules : a highly specific molecular and cytological combined predictor of malignancy

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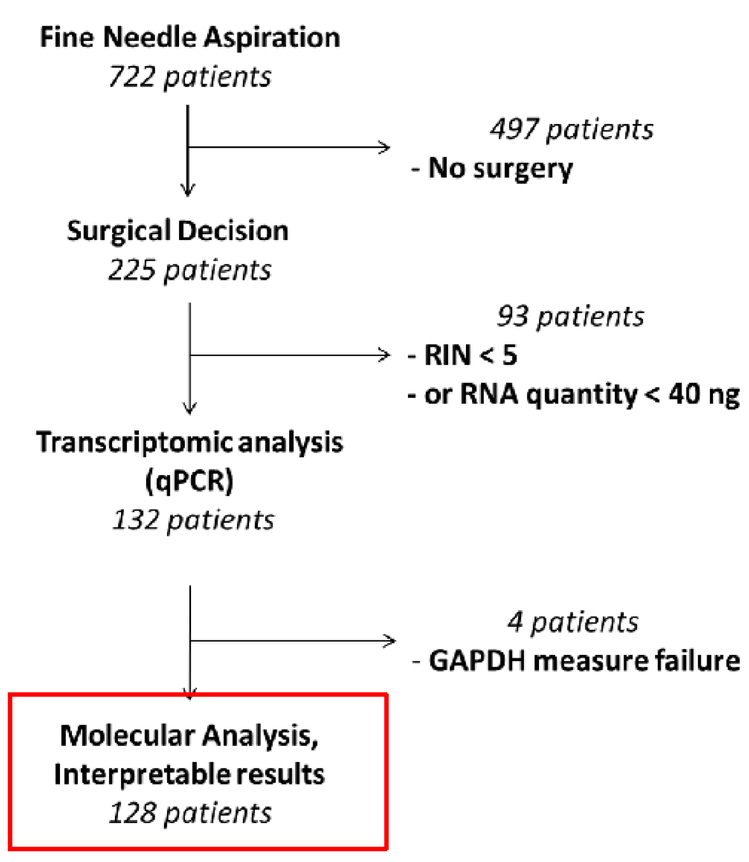
Context : Thyroid nodules prediction of malignancy is based on ultrasonographic examination and cytological analysis. Up to 30% of thyroid nodules remain "indeterminate" after cytological examination using Bethesda Classification.

Objective : This monocentric prospective study aimed to identify a molecular signature to improve the accuracy of preoperative diagnosis of nodules, taking into account the prevalence of the disease and the differential clinical consequences of false-negative and false-positive results.

Material

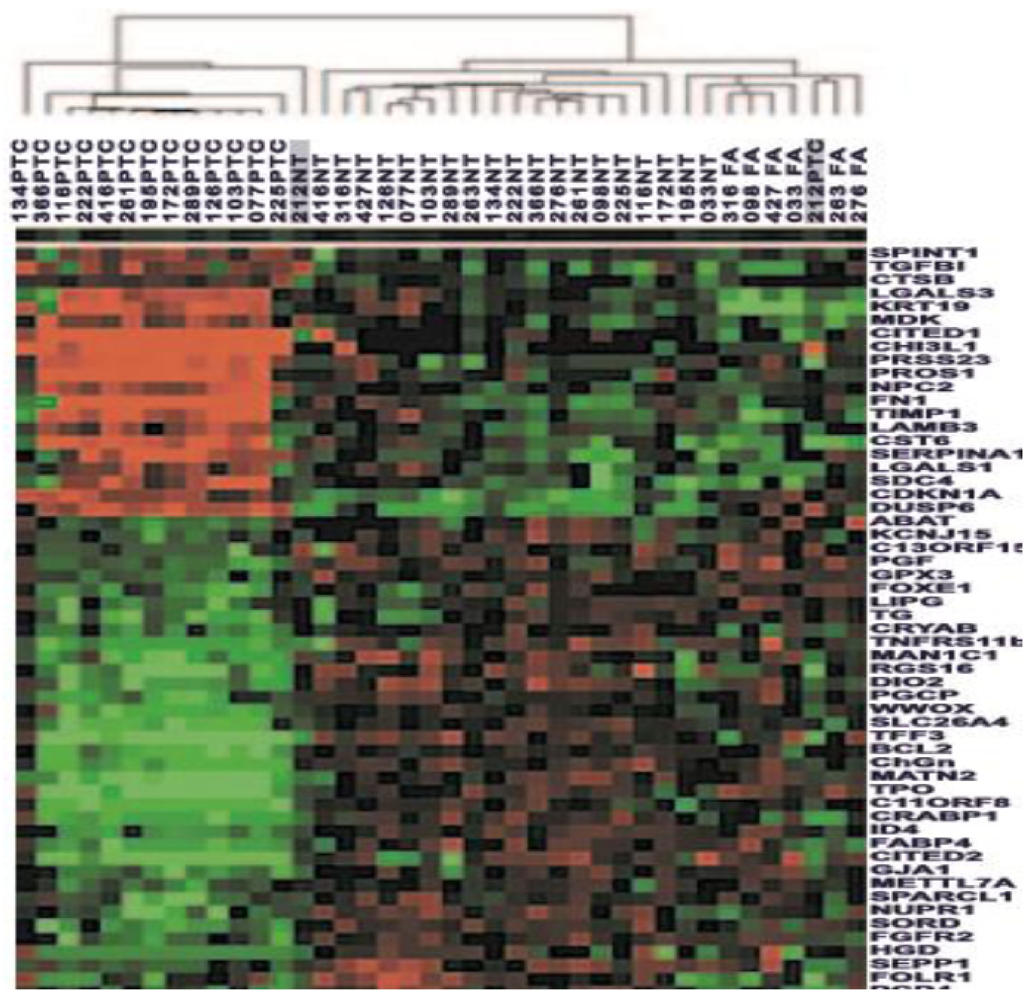


722 patients, prospectively included, underwent Fine-needle-Aspiration (FNA) for 1 cm or more thyroid nodule. One sample was kept frozen.

In case of surgical decision, based on clinician judgment, molecular analysis was performed.

The **Study Popuation** consisted of patients with available cytological (Bethesda classification), histological and molecular result.

Method : transcriptomic analysis



We performed a transcriptomic analysis of 20 genes selected from a previous study (1) using qPCR on FNA material.

A **logistic regression model** using genes expression levels as linear covariates generated the **molecular predictor**.

A **gene selection** was made using Boostrap method and Akaike information Criterion.

7 genes predicted the best malignancy

Gene	Adjusted OR [95% CI]	p-value *
FN1	2.01 [1.12; 3.58]	0.015
CITED2	0.31 [0.17; 0.58]	<0.001
CITED1	2.60 [1.45; 4.65]	<0.001
CHI3L1	0.50 [0.29; 0.87]	0.012
TFF3	0.46 [0.26; 0.82]	0.005
CDKN1A	1.71 [0.99; 2.98]	0.046
CSGALNACT1	1.79 [0.98; 3.28]	0.047

* log-likelihood ratio test

Results : cytology and histology

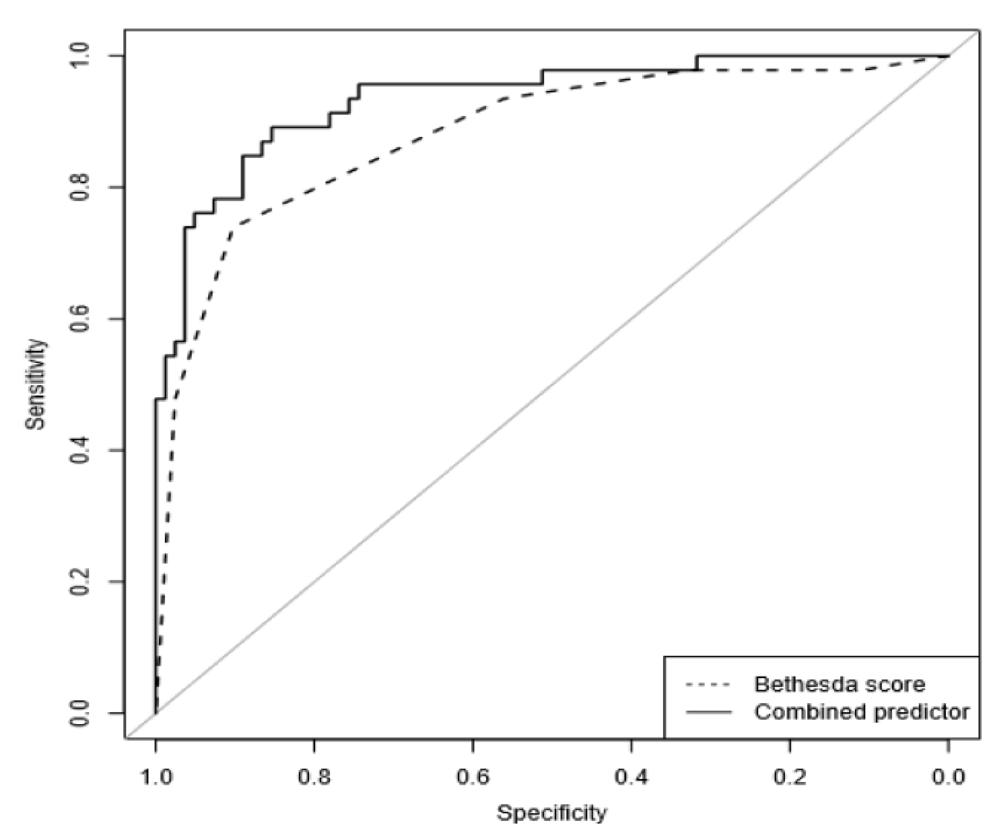
The malignancy prevalence among Bethesda categories was similar to the rates reported in literature (2).

Bethesda level	Malignant tumors in the study* frequencies	percent
I	1/10	10.0
II	0/18	0.0
III	2/21	9.5
IV	9/37	24.3
V	12/18	66.7
VI	22/24	91.7
Total	46/128	36.0

*** Malignant tumors (46)**

papillary thyroid carcinomas (39)
follicular thyroid carcinomas (3)
medullary thyroid carcinomas (2)
anaplastic thyroid cancer (1)
poorly differentiated carcinoma (1)

Results : combined predictor performances



A **logistic regression model** using two covariates, molecular result (7 genes) and cytological Bethesda category, generated **the combined predictor.**

The Aera under the curve of the combined predictor was **significantly higher than those of Bethesda classification** (DeLong test p = 0,004).

In **our data-set** (36% prevalence of malignancy), the combined predictor achieved **a high specificity :**

Sensitivity : 76,1 % [65.22; 97.83]
Specificity : 95,1 % [85.37; 100.00]

Results : clinical situations and performances

Thyroid cancer prevalence being around 7% among generic aspirated nodules (2), we optimized the performances **considering this prevalence and 2 different benefit-to-harm ratios.**

Thus, the combined predictor still harboured a high specificity together with an acceptable sensitivity.

Probability of malignant tumor*	Ratio*	Sensitivity	Specificity
In our dataset			
36%	4: 1	95.7% [86.96 ;100.00]	74.4% [66.43; 93.90]
36%	1: 1	76.1% [65.22; 97.83]	95.1% [85.37; 100.00]
36%	1: 4	73.9% [39.13; 86.96]	96.3% [95.12; 100.00]
In a virtual population			
7%	4: 1	73.9% [52.17; 89.13]	96.3% [92.04; 100.00]
7%	1: 1	47.8% [35.82; 82.61]	100% [98.78 ; 100.00]
7%	1: 4	47.8% [34.78; 71.74]	100% [100.00 ; 100.00]

* The combined predictor model gave a probability of malignancy. The cut-off probability to classify a nodule as malignant or non malignant was set taking into acount the malignancy prevalence.
Then, we introduced the benefit-to-harm ratio concept by weighting the number of false negative and false positive results.

Conclusion: We present the development of a **very specific molecular test** that may improve the pre-operative diagnosis of thyroid nodules. Moreover, its performances optimization according to the prevalence of the disease may avoid a reduction in its performances into clinical practice and enables an adaptation to the population of use.

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