Ultrasound-Guided Fine-Needle Aspiration of Thyroid Nodules: New events

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INTRODUCTION

• The assessment of thyroid nodules is a common clinical problem.
  • Thyroid nodules detected by ultrasound (US) : 67% of the adult population
  • Thyroid cancers :
    - 1% of all cancers – Most common endocrine cancer
    - 5% of thyroid nodules
    - Low mortality and morbidity
    - Global 10-years survival: > 90%

• **Challenge**: efficiently stratify patients according to their risk of malignancy in order to identify the best follow-up and therapeutic options

Yeung Oncologist 2008
DeLellis R et al., WHO 2004
Fine-needle aspiration (FNA)

• Predominant method used for the primary diagnosis of benign and malignant thyroid nodules
  – Categorisation of patients as operative or non-operative candidates
  – Intrinsic limitations:
    • **Distinguishing between benign and malignant follicular lesions**
    • Evaluation and treatment of patients with follicular proliferation cytology still remain problematic.

• Follicular Proliferation (FP):
  – Associated with a 20-30% incidence of malignancy
  – All patients referred to SURGERY

• We thus need to **improve the management** of patients with FP:
  – Clinical and US data that predict malignancy
  – Development of biomarkers

Yeung Oncologist 2008
Hegedus The New England Journal of Medicine 2004
Ultrasound (US) features:
- Multinodularity, echogenicity, size, solid or cystic, ca++, regional ADP

Clinical features:
- > 1cm or suspicious clinical features

Radionuclide scan
- (Hypofunction nodule)

Low cost effective method:
- 21-gauge needle
- 10-ml syringe
- Slices

Aspirated material smear on slices
- US probe perpendicular to the thyroid
  - (2-3 passes/nodule)

Cytological diagnosis
Follicular Proliferation < Follicular lesions

- Colloid nodules
- Well-differentiated tumours < follicular cells
  - Follicular adenoma
  - Follicular carcinoma
  - Papillary carcinoma

Follicular cells

Colloid

C-cell
Follicular proliferation

Cytological diagnoses:

Colloid nodule (AH) - Adenoma - Carcinoma
Adenoma versus Follicular Carcinoma Minimally Invasive

ADENOMA

FOLLICULAR CARCINOMA

Capsular effraction Vascular embole

Gal3

CK19
Papillary Carcinoma

• **WHO 2004:**

  “a malignant epithelial tumour showing evidence of *follicular cell differentiation* and characterized by *distinctive nuclear features*”
• Papillary pattern
• Increased nuclear size
• Clear nuclei
• Intranuclear inclusion
• Coffee bean aspect
Follicular Variant of Papillary Carcinoma

CK19

Gal3
• To evaluate the diagnostic value of FNA cytology

• To examine risk factors associated with malignancy
  – Grading system for Follicular Proliferation FNA diagnosis
  – Additive contribution of clinical and US features

• Refined decision aid tool for surgical indication integrating clinical, US and cytological features
Materials and Methods

• Retrospective analysis of 924 patients with FNA followed by surgery from 1986 to 2007 at Erasme Hospital.

• Clinical data collected for each patient:
  – age, sex, prior medical history, thyroid scan results, biochemical functional status, serum thyroglobulin level and thyroid antibodies (anti-thyroglobulin, anti-thyroid peroxidase or anti-TSH receptor antibodies)
US-guided FNA procedure

- Experimented pathologist
- US guidance by a radiologist
- US examination
  - multinodularity
  - nodule size
  - solid or cystic nodule +/- endocystic proliferation,
  - calcification
  - echogenicity, echogenicity pattern
  - regional lymphadenopathy (lymph node diameter >1 cm)
- Aspiration with a 21-gauge needle attached to a 10-mL syringe
- +/- 3 slides per aspiration: diff-quick stain
Cytological diagnosis

• **Unsatisfactory/Non-diagnostic samples:** poor fixation, poor cell preservation or hypocellularity

• **Benign diagnosis:** colloid nodules, colloid nodules with cystic changes and lymphocytic thyroiditis

• **Follicular proliferation (FP):**
  – **Grade 1 FP:** variable amounts of colloid, including some sheets of follicular cells presenting with low architectural atypia and without cellular atypia. The pathologist favoured a follicular neoplasm but a benign lesion could not be excluded.
  – **Grade 2 FP:** hypercellular specimens with scant colloid and sheets of follicular cells and with numerous atypical architectural features. The pathologist favoured a follicular neoplasm.
  – **Grade 3 FP:** applied to specimens with grade 2 FP criteria without a papillary pattern. Cytological features suggestive of papillary carcinoma, but not in sufficient quantity or quality for a definitive diagnosis of the follicular variant of papillary carcinoma.

• **Malignant diagnosis:** papillary carcinoma (PC), medullary carcinoma (MC), anaplastic carcinoma (AC), lymphoma and metastasis

→ **FP and Malignant diagnoses: Surgical indication**
Follicular Proliferation Grading

Follicular Proliferation
GRADE 1

- Variable amount of colloid
- Some sheets of follicular cells with low architectural atypia and without cellular atypia

• Follicular neoplasm but a benign lesion not excluded

Follicular Proliferation
GRADE 2

- Scant colloid
- Hypercellularity
- Sheets of follicular cells with numerous atypical architectural features

• Follicular neoplasm favoured

Follicular Proliferation
GRADE 3

- Grade 2 FP features
- No papillary pattern
- + Cytological features suggestive of PTC but not in sufficient quality or quantity for a definitive diagnosis of the follicular variant of PTC

• Malignant follicular neoplasm (FVPTC) suspected
Results: Histological diagnoses

- 924 patients:
  - 766 benign diseases
  - 15 well-differentiated follicular tumours with uncertain malignant potential (FT-UMP)
  - 143 cancers

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Benign</th>
<th>Malignant*</th>
<th>p-value**</th>
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<tbody>
<tr>
<td>Age (years; mean ± SEM)</td>
<td>46.1 ± 0.5</td>
<td>50.6 ± 1.3</td>
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<td>Sex ratio (F/M)</td>
<td>599/167</td>
<td>120/38</td>
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<td>Thyroid Functional Status</td>
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<td>Hypothyroidism</td>
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<td>Euthyroidism</td>
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<td>Hyperthyroidism</td>
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<tr>
<td>Thyroglobulin (ng/ml; mean ± SEM)</td>
<td>204 ± 19</td>
<td>602 ± 174</td>
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<td>Thyroid Antibodies (-/+ )</td>
<td>509/118</td>
<td>90/31</td>
<td>N.S.</td>
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<td></td>
<td>Benign</td>
<td>Malignant*</td>
<td>p-value**</td>
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<td><strong>Nodule characteristics</strong></td>
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<td>Radionuclide scan</td>
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<td>Hypofunctioning</td>
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<td><strong>Ultrasonographic Features</strong></td>
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<td>Unique/Multinodular</td>
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<td>Mean Size (mm, mean ± SEM)</td>
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<td>Echo structure</td>
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<td>Cystic++</td>
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<td>Cystic+</td>
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# Cytological-Histological Diagnoses Correspondence

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<th>Cytological diagnosis</th>
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<th>Benign</th>
<th>Adenoma</th>
<th>Cancer*</th>
<th>MNG</th>
<th>Benign**</th>
<th>AD</th>
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<th>PC</th>
<th>FV</th>
<th>PC</th>
<th>FC</th>
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<th>AC</th>
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<td>Grade 1</td>
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<td>23</td>
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<td>8</td>
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<td><strong>Cancer</strong></td>
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<td>Papillary C.</td>
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<td>2%</td>
<td>4%</td>
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<td>Anaplastic C. vs. metastasis</td>
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<tr>
<td>Lymphoma</td>
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<td>80%</td>
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<td><strong>Total</strong></td>
<td>924</td>
<td>62.5%</td>
<td>33.3%</td>
<td>4.2%</td>
<td>405</td>
<td>25</td>
<td>336</td>
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<td>52</td>
<td>44</td>
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<td>3</td>
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</table>
• Risk of malignancy:
  – 4.2% for unsatisfactory specimens
  – 1.6% for benign diagnosis
  – 19% for FP (G1: 7.7%; G2: 17.7%; G3: 45.7%)
  – 94.9% for cancers

• Regarding the surgical indication
  – FNA Sensitivity: 78.8% - Specificity: 80.6% - PPV: 82.5% - NPV: 76.7% - Global accuracy of 79.7%.

• Regarding the malignancy diagnosis
  – FNA Sensitivity: 91.5% - Specificity: 99.1% - PPV: 94.9% - NPV: 98.4% - Global accuracy of 97.8%.
FNAB & IMMUNOHISTOCHEMISTRY

- PTC diagnosis: Sb: 92%; Sp: 97%
- Benign vs malignant nodules: 29.5% vs 85%
- Malignancy in FP cytology: Sb: 78%; Sp: 93%

- Benign vs malignant nodules: 22.7% vs 92.6%
- Malignancy in FP cytology: Sb: 92% and Sp: 94%

Nasser SM, Cancer 2000
Saleh HA, Cytojournal 2009
Bartolazzi A, Lancet Oncol 2008

Saleh HA, Cytojournal 2009
Saggiorato E, Endocr Relat Cancer 2005
MOLECULAR ANALYSIS OF THYROID TUMORS

• Dramatic expansion of our understanding in molecular genetics of thyroid cancer
  - Better understanding of thyroid tumor biology
  - Ancillary tool for cytological and pathological diagnosis of thyroid cancer for better tumor prognostication

• Four major molecular alterations (mutually exclusive)
  - **BRAF** mutation (PTC: 35-70% (*tall cells & classic*); some ATC; no FTC, no benign thyroid nodules)
  - **RAS** mutation (PTC: 15-20% (*FVPTC*); FTC: 40-50%; Adenomas: 20-40%; few benign colloid nodules)
  - **RET/PTC** rearrangements (PTC: 10-20% (*Ret/PTC1: classic*); some adenomas and benign nodules)
  - **PAX8/PPARY** rearrangement (PTC<5% (*FVPTC*); FTC: 30-40%; Adenomas: 2-10%)
BRAF Mutation

• >95% of BRAF mutations:
  • T to A transversion: leading to a substitution of valine by glutamic acid at residue 600 of the protein (V600E)
  • Constitutive activation of BRAF kinase → Chronic stimulation of the MAPK pathway → Thyroid carcinogenesis

• 35-70% of Papillary Thyroid Carcinoma (PTC)
  • +++ Classic papillary (60%) and Tall cell variant (70%-80%) of PTC
  • Only 10% in the Follicular variant of PTC
  • Not found in Follicular carcinoma and benign thyroid nodules

• Correlates with aggressiveness of PTC
  • Extra-thyroidal extension
  • Increased tumour size
  • Advanced tumour stage at presentation
  • Lymph node or distant metastases
  • Reduced overall survival

Nikiforov Y. Modern Pathol 2011
Basolo F et al. J Clin Endocrinol Metab 2010
Elisei R et al., J Clin Endocrinol Metab 2008
**BRAF (V600E) DETECTION BY qPCR**

WT DNA  \[\text{WT primer} \rightarrow T \]  Mutated DNA  \[\text{mut primer} \rightarrow A \]  

Low $\Delta C_t = \text{Mutation}$  

High $\Delta C_t = \text{No mutation}$
A 67-years old women

FNAB:
Right nodule
23x31x30mm

PTC diagnosis

CK19 +
Gal-3 +

Thyroidectomy

Right Lobe

Left Lobe

Classic PTC

FVPTC

Low ΔCt = Mutation

Low ΔCt = Mutation

High ΔCt = No mutation
IHC:

CK19
Gal-3

Thyroid Nodule

US guided FNAB

• US features: unique, solid, hypoechoic nodule
• Radionuclide scan: hypofunctional nodule

Unsatisfactory (MP: 4.2%)

Benign diagnosis

MSG

GRADE 1 FP (MP: 7.7%)

GRADE 2 FP (MP: 17.7%)

GRADE 3 FP (MP: 45.7%)

Malignant diagnosis

PTC suspected

PTC diagnosis

Diagnostic BRAF test

Prognostic BRAF test

>1cm

Or Suspicious clinical features
CONCLUSIONS

- FNAB = Gold standard
- Limitation: Follicular proliferation
- Necessity
  - To grade Follicular Proliferation
  - To develop Multivariate risk model of malignancy
- Prospective study included biomarkers
  - IHC: galectin-3 and CK19
  - *BRAF* status