FNA cytology of the thyroid

Gabrijela Kocjan, FRCPath
Head of Diagnostic Cytopathology
University College London
Cytopathology of the thyroid

• The bigger picture
• Bird’s eye view of FNA diagnosis
• Classify we must
• Do we agree? Actual or virtual slides?
• What is non-diagnostic? Atypical?
• Are cysts benign or non-diagnostic?
• When to repeat and how many times?
• Will genes help?
The most commonly occurring papillary thyroid cancer in the United States is now a microcarcinoma in a patient older than 45 years

- Estimated cases of thyroid cancer in USA – 2010:
  - New cases: 44,670
  - Deaths: 1,690
- SEER database 1974-2006
  - Diagnosis of PTC has shifted 30yrs – 40-50 yrs
    - Until 1999 < 45 yrs
    - After 1999 > 45 yrs
  - Largest increase in <1.0 cm PTC
- Increase in small tumor detection but presentation at higher stage has doubled

Hughes et.al. Thyroid 2011
Ultrasound guided FNA

Information needed

- Full clinical details
- Details of the aspiration procedure
- The site of the abnormality
- The site of sampling

ROSE in one stop clinic ideal but not always possible

Make technically optimal samples
# Cancer Rates for Solitary and Multiple Thyroid Nodules

<table>
<thead>
<tr>
<th>Definition of nodularity</th>
<th>FNA technique</th>
<th>Cancer rate</th>
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<tr>
<td></td>
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<td>1 nodule</td>
</tr>
<tr>
<td>McCall/USA</td>
<td>scan/histo</td>
<td>17%</td>
</tr>
<tr>
<td>Belfiore/Italy</td>
<td>scan</td>
<td>5%</td>
</tr>
<tr>
<td>Cochand/France</td>
<td>scan/US</td>
<td>13%</td>
</tr>
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<td>Sachamedchi/USA</td>
<td>scan</td>
<td>8%</td>
</tr>
<tr>
<td>Marqusee/USA</td>
<td>US</td>
<td>7%</td>
</tr>
<tr>
<td>Papini/Italy</td>
<td>US</td>
<td>9%</td>
</tr>
<tr>
<td>Barroeta /USA</td>
<td>US</td>
<td>52%</td>
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## Cancer Rates for Solitary and Multiple Thyroid Nodules

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</tr>
</tbody>
</table>
Aim of FNA thyroid:
To reduce surgery for benign nodules

Patient with thyroid nodule

Excision

No excision
Bird’s eye view of thyroid FNA

Cell /colloid ratio is a crucial factor in deciding the nature of the lesion.
colloid

cells
colloid

cells
cells

400 μm
<table>
<thead>
<tr>
<th>Description</th>
<th>RCPPath/BTA (UK)</th>
<th>BSRTC (Bethesda) USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diagnostic for cytological diagnosis</td>
<td>Thy1</td>
<td>I.</td>
</tr>
<tr>
<td>Cystic lesion (Thy1c)</td>
<td></td>
<td>Cyst fluid only</td>
</tr>
<tr>
<td>Non-neoplastic/benign</td>
<td>Thy2</td>
<td>II.</td>
</tr>
<tr>
<td>Thy2c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colloid cyst</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoplasm possible / Atypia of undetermined significance</td>
<td>Thy3a</td>
<td>III.</td>
</tr>
<tr>
<td>Neoplasm possible/ suggesting follicular neoplasm</td>
<td>Thy3f</td>
<td>IV.</td>
</tr>
<tr>
<td>Thy3f</td>
<td></td>
<td>Follicular neoplasm or suspicious for a follicular neoplasm or Hürthle cell (oncocytic) type</td>
</tr>
<tr>
<td>Suspicious of malignancy</td>
<td>Thy4</td>
<td>V.</td>
</tr>
<tr>
<td>Malignant</td>
<td>Thy5</td>
<td>VI.</td>
</tr>
</tbody>
</table>
1. Agreement about the need for standardization of thyroid FNA

2. The majority favoured a translation of the local terminology to Bethesda as the first step towards a unified nomenclature

Non-diagnostic—BETHESDA I (Thy 1)

- Not of adequate epithelial cellularity
  - FNA of solid lesions should have at least six groups of 10 thyroid follicular epithelial cells across all the submitted slides
- Cystic lesion fluid specimens which do not reach the epithelial cell adequacy criterion, contain macrophages but without abundant colloid (Thy 1c)
Non-diagnostic – BETHESDA I (Thy 1)
Non-neoplastic – Bethesda II (Thy 2)

- Normal thyroid tissue
- Thyroiditis
- Hyperplastic nodules
- Colloid nodules (6 groups of 10 cells)
- Cystic fluid with adequate epithelium
- Cystic fluid with colloid and macrophages, Thy 2c
Atypia of uncertain significance—Follicular lesion of uncertain significance—BETHESDA III (AUS/FLUS) (Thy3A)

- Architectural ‘atypia’
- Sparse colloid
- Sparsely cellular samples
- Focal cytological changes
- A compromised specimen
- Atypical ‘cyst lining cells’
Follicular neoplasm possible-BETHESDA IV (Thy 3f)

- Follicular hyperplasia
- Follicular adenoma
- Oncocytic adenoma
- Follicular adenoma
- Follicular carcinoma
Suspicious of malignancy –
BETHESDA V (Thy4)

Suspicious of malignancy, features do not allow confident diagnosis of malignancy

Thy 4 reports should be discussed at the thyroid multidisciplinary meetings
Suspicious of malignancy – BETHESDA V (Thy4)

Lipid rich variant of follicular carcinoma

Specimens of low cellularity and mixed cell types (normal and atypical)
The tumour type suspected should be clearly stated, and will often be papillary carcinoma

FVPC
Suspicious of malignancy
BETHESDA V (Thy4)

Hyalinising trabecular adenoma
Follicular variant of papillary carcinoma
Papillary carcinoma
Malignant – BETHESDA VI (Thy5)

Thy 5 reports should be discussed at the thyroid multidisciplinary meetings

- Papillary ca
- Medullary ca
- Anaplastic ca
- Lymphoma
The Interobserver Reproducibility of Thyroid Fine-Needle Aspiration Using the UK Royal College of Pathologists’ Classification System

Gabrijela Kocjan, MD, FRCPATH,1 Ashish Chandra, MD, FRCPATH,2 Paul A. Cross, FRCPATH,3 Thomas Giles, MB, ChB, FRCPATH,4 Sarah J. Johnson, MB BS, PhD, FRCPATH,5 Timothy J. Stephenson, MD, FRCPATH,6 Michael Roughton, MSc,7 and David N. Poller, MD, FRCPATH8

Key Words: Thyroid; Fine-needle aspiration; FNA; Bethesda; Classification; Interobserver reproducibility; Cytology

- 6 observers, all experienced in thyroid FNA, members of RCPath working group
- 200 thyroid FNA slides from routine practice
- Circulated by post to each participant
- 1 or 2 slides per case
- Results collated
Interobserver Agreement for THYROID FNA reporting using UK RCPAth classification

Interobserver Agreement for Combined Reporting Categories relative to clinical management

Conservative management BTSRTC 1-3

Surgical management TBSRTC 4-6

- Distinguishing non-neoplastic and neoplastic disease
- The use of FNA classification is very helpful in providing clarity in clinical management of thyroid lesions
Comparison between the **microscopic** and **digital** interobserver agreement in reporting thyroid cytology
The UK Royal College of Pathologists Thyroid Fine-Needle Aspiration Diagnostic Classification Is a Robust Tool for the Clinical Management of Abnormal Thyroid Nodules

Claudia Lobo, Andrew McQueen, Tim Beale, Gabrijela Kocjan

- Benign, 69%
- Indeterminate, 20%
- Malig, 7%
- Unsatisfactory, 3%

- follicular, 14%
- atyp/susp, 6%
<table>
<thead>
<tr>
<th>UK RCPPath</th>
<th>BETHESDA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnostic category</strong></td>
<td><strong>Risk of cancer (%)</strong></td>
</tr>
<tr>
<td>Thy1/Thy1c</td>
<td>Non-diagnostic for cytological diagnosis</td>
</tr>
<tr>
<td>Thy2/Thy2c</td>
<td>Non-neoplastic (/) /Benign</td>
</tr>
<tr>
<td>Thy 3a</td>
<td>Neoplasm possible – atypia/non-diagnostic /Atypia of undetermined significance or follicular lesion of undetermined significance</td>
</tr>
<tr>
<td>Thy3f</td>
<td>Neoplasm possible - suggesting follicular neoplasm /Follicular neoplasm or suspicious for a follicular neoplasm</td>
</tr>
<tr>
<td>Thy 4</td>
<td>Suspicious of malignancy</td>
</tr>
<tr>
<td>Thy5 Malignant</td>
<td></td>
</tr>
</tbody>
</table>
### PPV of malignant diagnosis for different thyroid FNA reporting categories

<table>
<thead>
<tr>
<th>Reporting Category</th>
<th>Total number with F/U available</th>
<th>Neoplastic (adenoma or carcinoma)</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thy 3a</td>
<td>10</td>
<td>50%</td>
<td>40%</td>
</tr>
<tr>
<td>Thy 3f</td>
<td>47</td>
<td>53%</td>
<td>36%</td>
</tr>
<tr>
<td>Thy 4</td>
<td>9</td>
<td>77%</td>
<td>66%</td>
</tr>
<tr>
<td>Thy 5</td>
<td>33</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>UK RCPATH</th>
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<tr>
<td><strong>Diagnostic category</strong></td>
<td><strong>Risk of cancer (%)</strong></td>
</tr>
<tr>
<td>Thy1/Thy1c</td>
<td>?</td>
</tr>
<tr>
<td>Non-diagnostic for cytological diagnosis Unsatisfactory</td>
<td></td>
</tr>
<tr>
<td>Thy2/Thy2c</td>
<td>?</td>
</tr>
<tr>
<td>Non-neoplastic ()/Benign</td>
<td></td>
</tr>
<tr>
<td>Thy 3a</td>
<td>40*</td>
</tr>
<tr>
<td>Neoplasm possible – atypia/non-diagnostic Atypia of undetermined significance follicular lesion of undetermined significance</td>
<td></td>
</tr>
<tr>
<td>Thy3f</td>
<td>28</td>
</tr>
<tr>
<td>Neoplasm possible - suggesting follicular neoplasm /Follicular neoplasm or suspicious for a follicular neoplasm</td>
<td></td>
</tr>
<tr>
<td>Thy 4</td>
<td>64</td>
</tr>
<tr>
<td>Suspicious of malignancy</td>
<td>64 f/u not available in more than half</td>
</tr>
<tr>
<td>Thy5</td>
<td>100</td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
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Comparison of thyroidectomies in 2005 and 2010

- **Benign lesions**
  - 2005-2006: 58.93%
  - 2010-2011: 47.22%

- **Malignant lesions**
  - 2005-2006: 41.07%
  - 2010-2011: 52.78%
Proportion of malignant lesions diagnosed preoperatively in 2005 and 2010

Cytological diagnosis

Malignant lesions

2005-2006

2010-2011

23
38
3
32
Thyroid FNA and Adequacy

– Frequently low cellularity samples
  • Cystic lesions
  • Poor quality sampling by inexperienced aspirators
  • Vascular targets - sample prone to dilution by blood
  • Colloid rich lesions

– Well differentiated carcinomas are composed of cells similar in appearance to “benign” follicular cells
Cystic change

Colloid goitre

Papillary ca
Goal of Adequacy Assessment

- Reduce “false negative” diagnoses arising from insufficient sampling

  - If adequacy criteria work:
    - Malignancy rate in “Inadequate” cases Greater than Malignancy rate in “Adequate” / Benign cases

  - If adequacy criteria fail:
    - Malignancy rate in “Inadequate” cases Equal to or less than Malignancy rate in “Adequate” / Benign cases
Epithelial Quantitation

• Adequacy is defined by a number of epithelial cells
  – Less than a magical number inadequate
  – More than a magical number adequate

• On a conceptional level, this seems logical
  – But in practice, it has limitations
Epithelial Quantitation

• Most commonly quoted criterion:

“at least 6 groups of benign follicular cells are required, each group composed of at least 10 cells.”

• Is referenced to:
Epithelial Quantitation

- Goellner et al. *Acta Cytol* 1987;31:587-590 (Mayo Clinic group)
  - Palpation guided
    - 25 G needle / 1-4 passes / direct smears (fluid – Millipore filter) / alcohol fixed / Pap stained
    - “Adequate” if 5 to 6 groups of 10+ epithelial cells
      - Colloid without cells is “non-diagnostic”
      - “To be considered adequate for interpretation, our rule of thumb requires five to six groups......”
  - 6,346 FNA in study
  - 21% “non-diagnostic” (1,299 cases)
    - 9.1% “non-diagnostic” excised (118 cases)
    - 8.5% of the cases excised had carcinoma on biopsy (10 cases)
      - 0.8% of “non-diagnostic” had carcinoma on biopsy considering all cases
  - 65% “benign” (4,103 cases)
    - 3.2% “benign” excised (130 cases)
    - 6.2% of the cases excised had carcinoma on biopsy (8 cases*)
      - 0.2% of “benign” had carcinoma on biopsy considering all cases

* 5 papillary ca, 1 Hürthle cell ca, 1 parathyroid ca, 1 lymphoma
Epithelial Quantitation

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* 5 papillary ca, 1 Hürthle cell ca, 1 parathyroid ca, 1 lymphoma
When to report *Thy 1c* and *Thy 2c*

‘the sample is in keeping with fluid from a cyst but there are no epithelial cells or colloid to confirm cyst type’.  

*Thy1c*

The sample is in keeping with fluid from a cystic colloid nodule but there are no/too few epithelial cells for confirmation’ *Thy2c*

Risk of malignancy 5 to 37% (estimated mean 15%)  
Majority are papillary carcinomas
Should cyst contents lacking epithelial cells be considered adequate?

– Features that **DO NOT** distinguish benign from malignant
  • Amount of cyst fluid
  • Macroscopic (gross) appearance of fluid
  • Presence of macrophages, blood, protein, “inflammatory” cells (cyst contents)

– Features that **DO** distinguish benign from malignant
  • **Abnormalities in epithelial cells**
    Jaragh et. al. *Cancer Cytopathol.* 2009;117:305-310
Is there a difference between acellular FNA and FNA with cyst contents?

- Histologic follow-up on non-diagnostic thyroid FNA from complex thyroid cysts
  - 11.1% rate of malignancy on non-diagnostic FNA from complex cysts
    - Acellular FNA (n=15) – 6.6%
    - Cyst contents FNA (n=21) – 14.3%

Does the presence of colloid define an FNA as adequate?

  • *Colloid without cells is “non-diagnostic”*
– The Bethesda system
  • “*Abundant colloid*” lacking epithelial cells is benign
    – When is it abundant?
    – When is it colloid? - Problem with liquid based preparations
      » Loss of colloid through the filter
      » Less easily recognized
How soon to repeat FNA?

The rationale for waiting 3 months is based on the unproven concern that the interpretation of an aspiration obtained after a shorter interval might be confounded by an exuberant repair reaction.

- No difference in false-positive interpretations between early and late repeats


- Repeat aspiration for cystic ND nodules is only recommended for those lesions with concerning ultrasonographic features

The management of the patient with a repeatedly non-diagnostic thyroid FNA

**How many times should FNA be repeated?**

**Repeatedly Nondiagnostic Thyroid Fine-Needle Aspirations Do Not Modify Malignancy Risk**

Vickie Y. Jo<sup>a</sup>  Paul A. VanderLaan<sup>a</sup>  Ellen Marqusee<sup>b</sup>  Jeffrey F. Krane<sup>a</sup>

57/834 UNDERWENT SURGERY

21% Malignancy identified histologically after a single NON DIAG FNA

20% After **2 or more** repeatedly NON DIAG FNA

Jo,VY.Acta Cytologica 2011;55:539–543
ATYPIA OF UNCERTAIN SIGNIFICANCE – BETHESDA III (AUS/FLUS) (Thy3A)

Probability of a malignant diagnosis 6–48%

0.7-18%

Targets for AUS/FLUS stated in the BSRTC guidelines

incidence (<7%)
risk of malignancy (5–15%)

Review of the literature revealed institutional differences in:
  • technical aspects
  • interpretation and application of criteria
  • analysis of outcome data
  • clinicopathologic interactions

Differential diagnosis of thyroid nodules using FNAC and oncogene mutation screening: Are we ready?

Lesions of ‘uncertain’ nature
– ‘suspicious for malignancy’
– ‘suspicious for follicular neoplasm,
– ‘suspicious for oncocytic neoplasm’
– ‘follicular lesions of undetermined significance (FLUSs)/atypia of undetermined significance

# Specificity of BRAF Detection in Thyroid FNAC

**Direct nucleotide (Sanger) sequencing**

<table>
<thead>
<tr>
<th>Samples (n)</th>
<th>BRAF positive</th>
<th>Final diagnosis in BRAF-positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid nodule FNA, prospective studies</td>
<td>1814</td>
<td>159</td>
</tr>
<tr>
<td>Thyroid nodule FNA, retrospective studies</td>
<td>685</td>
<td>291</td>
</tr>
<tr>
<td>Research FNA of surgically removed thyroid</td>
<td>267</td>
<td>131</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2766</strong></td>
<td><strong>58</strong></td>
</tr>
</tbody>
</table>

Marina N. Nikiforova and Yuri E. Nikiforov. Molecular Diagnostics and Predictors in Thyroid Cancer. THYROID Volume 19, Number 12, 2009
Combined cytology and molecular testing

- Cytological diagnosis of PTC alone 56/90 (62.3%)
- BRAF V600E mutation/ cytology suspicious 8/32 (56.2%)
- Cytology combined with BRAF V600E 74/90 (82.2%)

Combined cytology and molecular testing

- Molecular testing
  - 60% sensitive for malignancy

- Cytology
  - 100% specific for malignancy
  - 40% sensitive for malignancy

- Molecular analysis and cytology
  - 80% sensitivity

RAS, RET/PTC, and PAX8/PPARgamma mutations also contribute substantially to cancer diagnosis

- In addition to BRAF mutation, which has been studied most extensively, detection of RAS, RET=PTC, and PAX8=PPARg mutations also contribute substantially to cancer diagnosis.

Marina N. Nikiforova and Yuri E. Nikiforov. Molecular Diagnostics and Predictors in Thyroid Cancer. THYROID Volume 19, Number 12, 2009
Correlation between results of cytology, molecular biology on cytological samples, and final histology

*Cantara, S. et al. J Clin Endocrinol Metab. 2010;95:1365-1369*
Molecular analysis of thyroid nodules

- BRAF, RAS, RET/PTC, and PAX8/PPARg mutations (correlation with cytology, surgical pathology, and clinical follow-up)

- The presence of a mutation was a strong indicator of malignancy with specificity of almost 100%

- BRAF, RET/PTC, and PAX8/PPARg mutations were always associated with carcinomas

- RAS mutations were found in FA in a few cases, but never in hyperplastic nodules

Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer

The American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer


ORIGINAL STUDIES, REVIEWS, AND SCHOLARLY DIALOG

THYROID CANCER AND NODULES

ATA Guidelines

Indeterminate cytology (follicular or Hurthle cell neoplasm, follicular lesion of undetermined significance, atypia). Indeterminate cytology, reported as “follicular neoplasm” or “Hurthle cell neoplasm” can be found in 15–30% of FNA specimens (4) and carries a 20–30% risk of malignancy (42), while lesions reported as atypia or follicular lesion of undetermined significance are variably reported and have 5–10% risk of malignancy. Clinical features such as male sex and nodule older patient age (67), or cytologic features of atypia (68) can improve the diagnostic accuracy in patients with indeterminate cytology. Markers are still low. Many molecular markers (cytokeratin, BRAF) have been evaluated to increase diagnostic accuracy for indeterminate nodules (70–87). Prospective studies have confirmed the ability (BRAF, Ras, RET/PTC) and protein markers to increase preoperative diagnostic accuracy for indeterminate thyroid nodules (69,73,74). Many molecular markers will be used in the future to optimize management of patients with indeterminate cytology on FNA specimens. Recently, 18F-FDG-PET scanning has been utilized in an effort to distinguish those indeterminate nodules that are benign from those that are malignant (75–78). 18F-FDG-PET scans appear to have relatively high sensitivity for malignancy but low specificity, but results vary among studies (79).

RECOMMENDATION 8

(a) The use of molecular markers (e.g., BRAF, RAS, RET/PTC, Pax8-PPAR, or galectin-3) may be considered for patients with indeterminate cytology on FNA to help guide management. Recommendation rating: C
**BRAF** Mutation Testing of Thyroid Fine-Needle Aspiration Specimens Enhances the Predictability of Malignancy in Thyroid Follicular Lesions of Undetermined Significance

Adebowale J. Adeniran  Pei Hui  David C. Chhieng  Manju L. Prasad  Kevin Schofield  Constantine Theoharis

Department of Pathology, Yale University School of Medicine, New Haven, Conn., USA

Indeterminate thyroid FNA

- **BRAF** -ve → Repeat FNA in 6–12 months
- **BRAF** +ve → Total thyroidectomy ± central lymph node dissection

---

**Sensitivity** 59.3%
**Specificity** 100%
**PPV** 100%
**NPV** 65.6%

**BRAF Mutation Analysis of Fine-Needle Aspiration Biopsies of Papillary Thyroid Carcinoma: Impact on Diagnosis and Prognosis**

Agnes Colanta  Oscar Lin  Laura Tafe  Ronald Ghossein  Khedoudja Nafa  
Talia Mitchell  Marc Ladanyi  Maria Arcila  
Memorial Sloan-Kettering Cancer Center, New York, N.Y., USA

Correlation of *BRAF status and PTC histology*

<table>
<thead>
<tr>
<th>BRAF status</th>
<th>Classical (n = 30)</th>
<th>Tall cell (n = 6)</th>
<th>Follicular (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>BRAF (+)</em></td>
<td>17 (57%)</td>
<td>5 (83%)</td>
<td>2 (12%)</td>
</tr>
<tr>
<td><em>BRAF (-)</em></td>
<td>13 (43%)</td>
<td>1 (7%)</td>
<td>14 (88%)</td>
</tr>
</tbody>
</table>

‘...it contributes little to reducing equivocal cytomorphic findings ...’

• none of AUS/FLUS cases BRAF pos
• 3 of 17 suspicious for malignancy

A variety of methods to detect the presence of *BRAF* mutations

- direct sequencing
- single-strand conformational polymorphism analysis
- dual priming oligonucleotide-based multiplex PCR direct DNA sequencing
- PCR-restriction fragment length polymorphism
- LightCycler PCR with allele-specific fluorescent probe melting curve analysis
- pyrosequencing

Example of a lesion yielding a **negative** result by standard sequencing but a **positive** result by LNA-PCR sequencing

Commercial test

Bethesda system

Benign

Indeterminate

AUS/FLUS
Neoplasm
Suspicious

Malignant
Nondiagnostic

Molecular classifier

Benign

Suspicious
The method detects 0.01% BRAF mutant DNA in the presence of 99.99% WT DNA.
Metabolomic profiles from tissue, particularly from FNAB cytology samples, have the potential to be used in conjunction with current diagnostics to help guide the clinical management of patients with thyroid nodules.
Conclusions

• Cell colloid ratio most important in dg
• Classifications are reproducible, actually and virtually
• Aim is less surgery for benign disease
• Atypical/FLUS should be kept at <7%
• 15% cysts are malignant
• Repeated non diagnostic samples do not exclude malignancy
• Molecular tests will help but they need to be standardised