How to Handle Thyroid FNA

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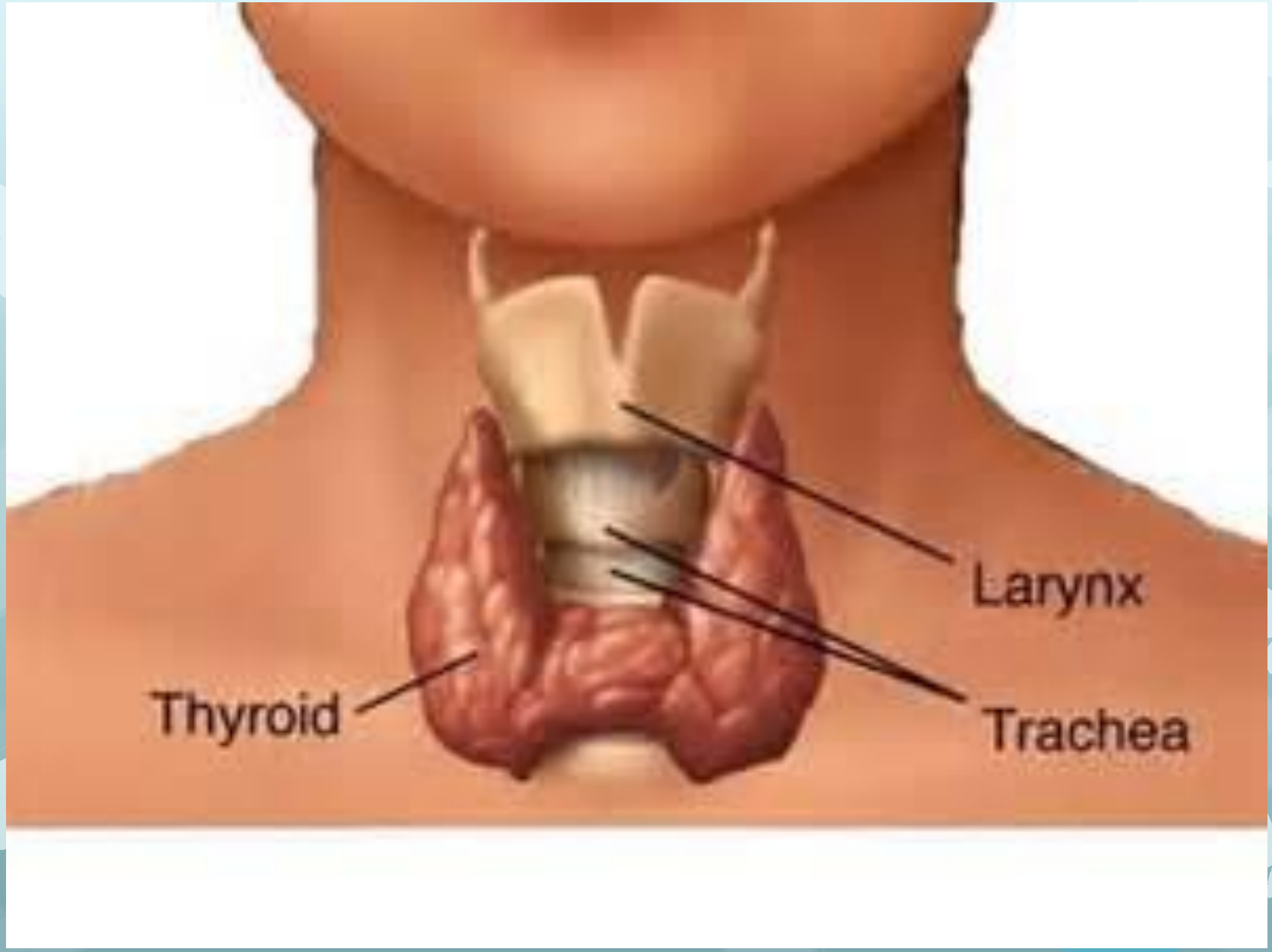
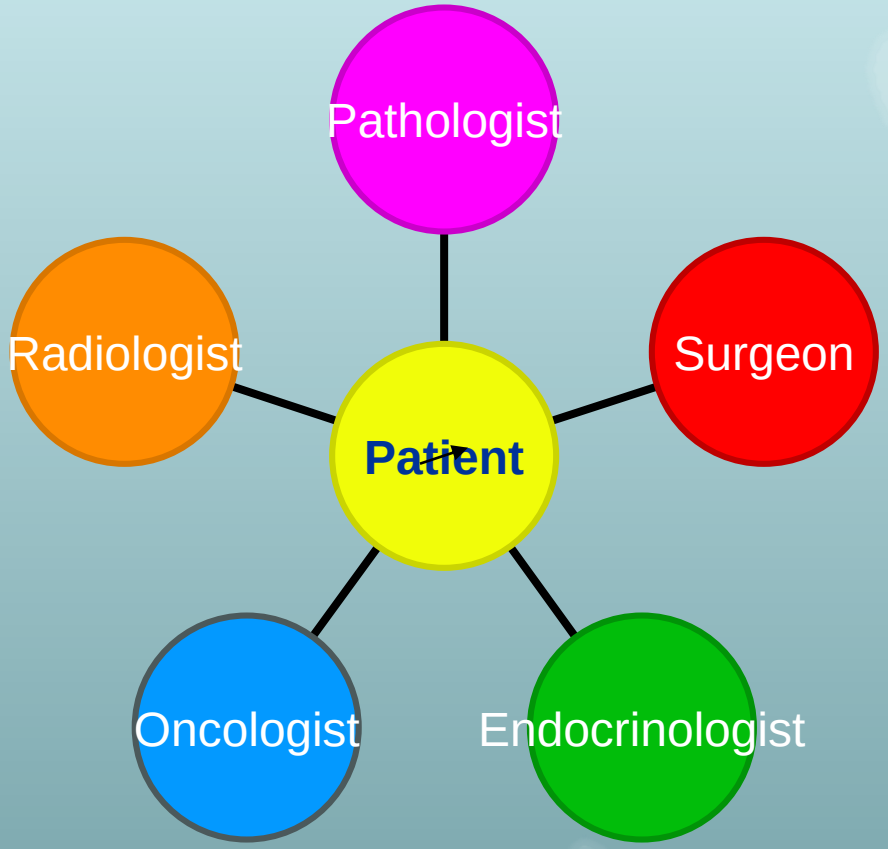
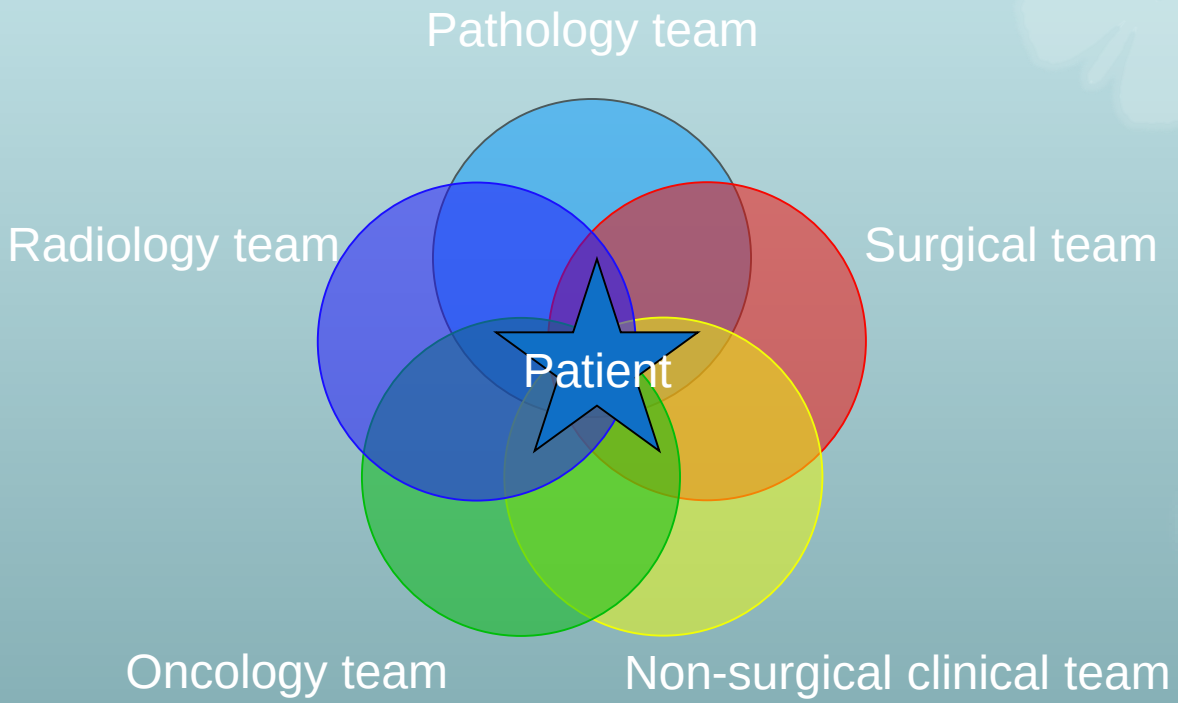

Larynx
Thyroid
Trachea

Compartmental Model



Multidisciplinary Model



Who should perform Thyroid FNA?

- Radiologists
- Endocrinologists
- Interventional Clinician Cytopathologists

Is a Cytopathologist competent enough to perform ultrasound-guided FNA ?

Comparative Studies of 300 Head and Neck FNAs Performed by Non-pathologists and a Cytopathologist with and without Ultrasound Guidance

- *Evidence for improved diagnostic value with ultrasound guided FNA performed by a Cytopathologist*

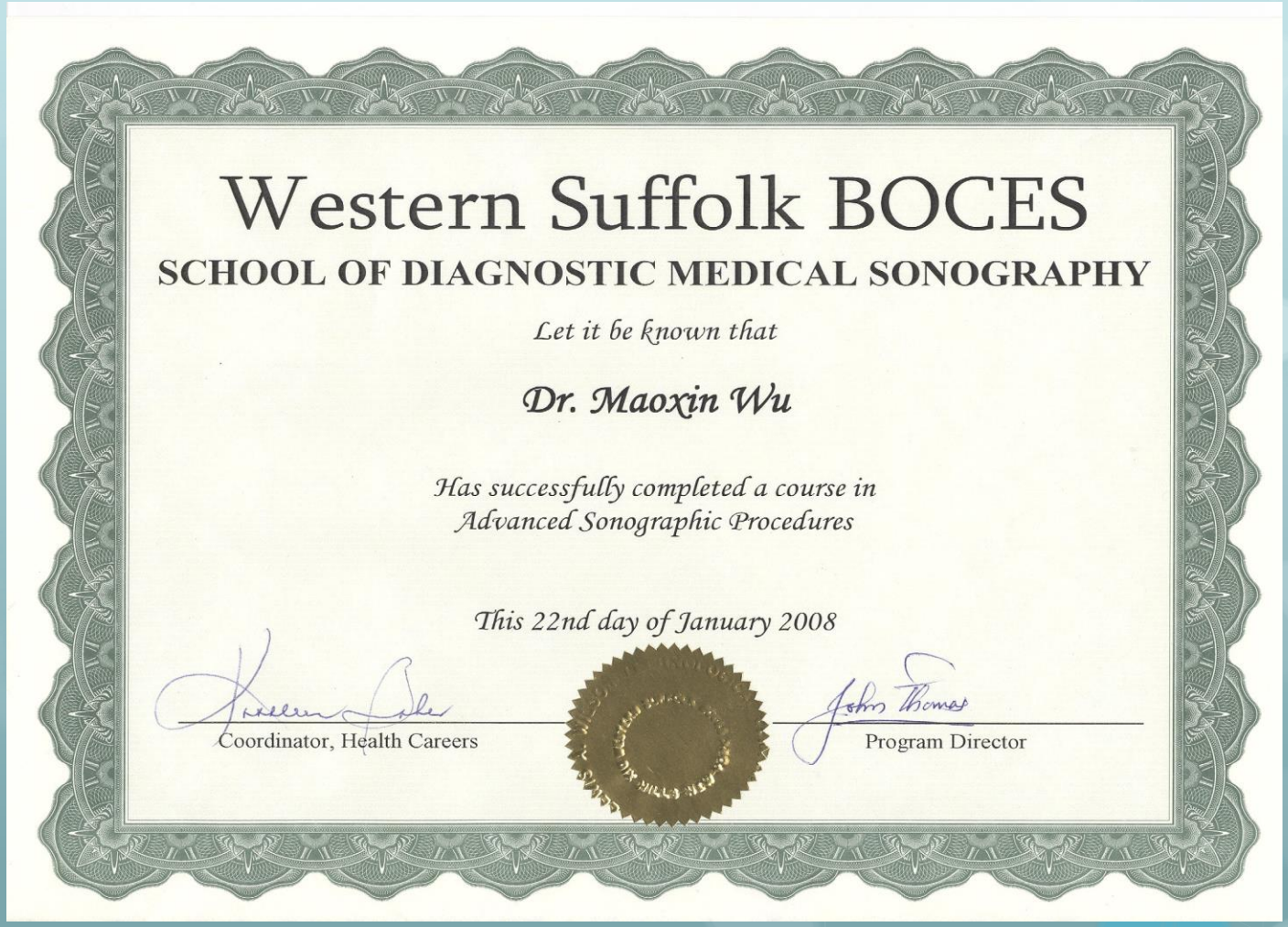
Diagnostic Rates of FNAs

FNA type	Non-Path FNA (N=100)	Path-PGFNA (N=100)
Diagnostic rate (%)	24	83
Suspicious/atypical suggestive/ non-specific rate (%)	43	10
Non-diagnostic rate (%)	33	7

Wu M, Burstein DE, Yuan S, Nurse LA, Szporn AH, Zhang D, Genden E. A comparative study of 200 fine needle aspiration biopsies performed by clinicians and cytopathologists. Laryngoscope. 2006 Jul;116(7):1212-5.

Statistics	**Non-Path FNA (N=100)**	**PGFNA (N=100)**
Sensitivity (%) (TP/TP+FN):	67	96
Specificity (%) (TN/TN+FP)	0 *	0 *
PPV (%) (TP/TP+FP)	100	98
NPV (%) (TN/TN+FN)	0 *	0 *

Qualification /Certification



Western Suffolk BOCES

SCHOOL OF DIAGNOSTIC MEDICAL SONOGRAPHY

Let it be known that

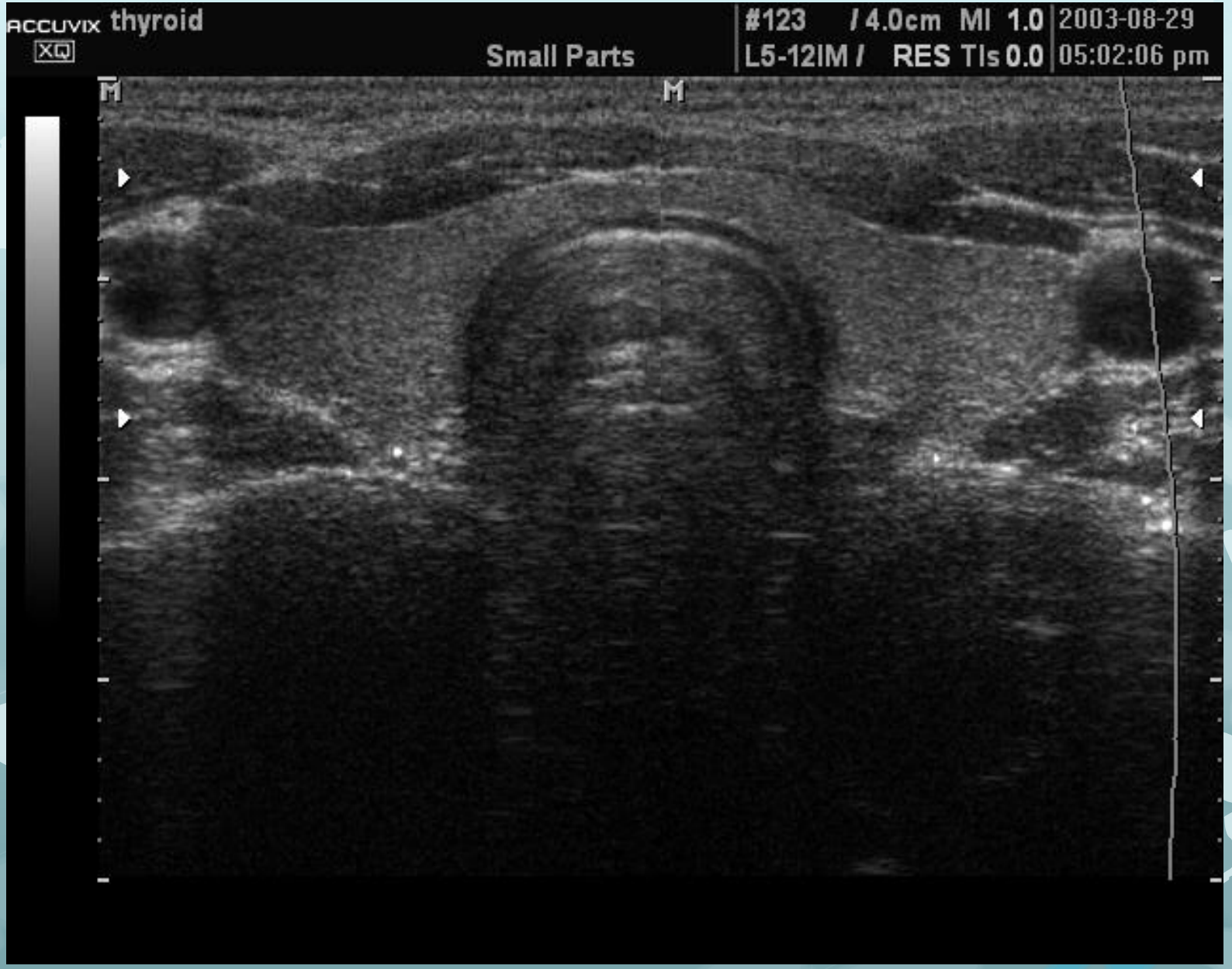
Dr. Maoxin Wu

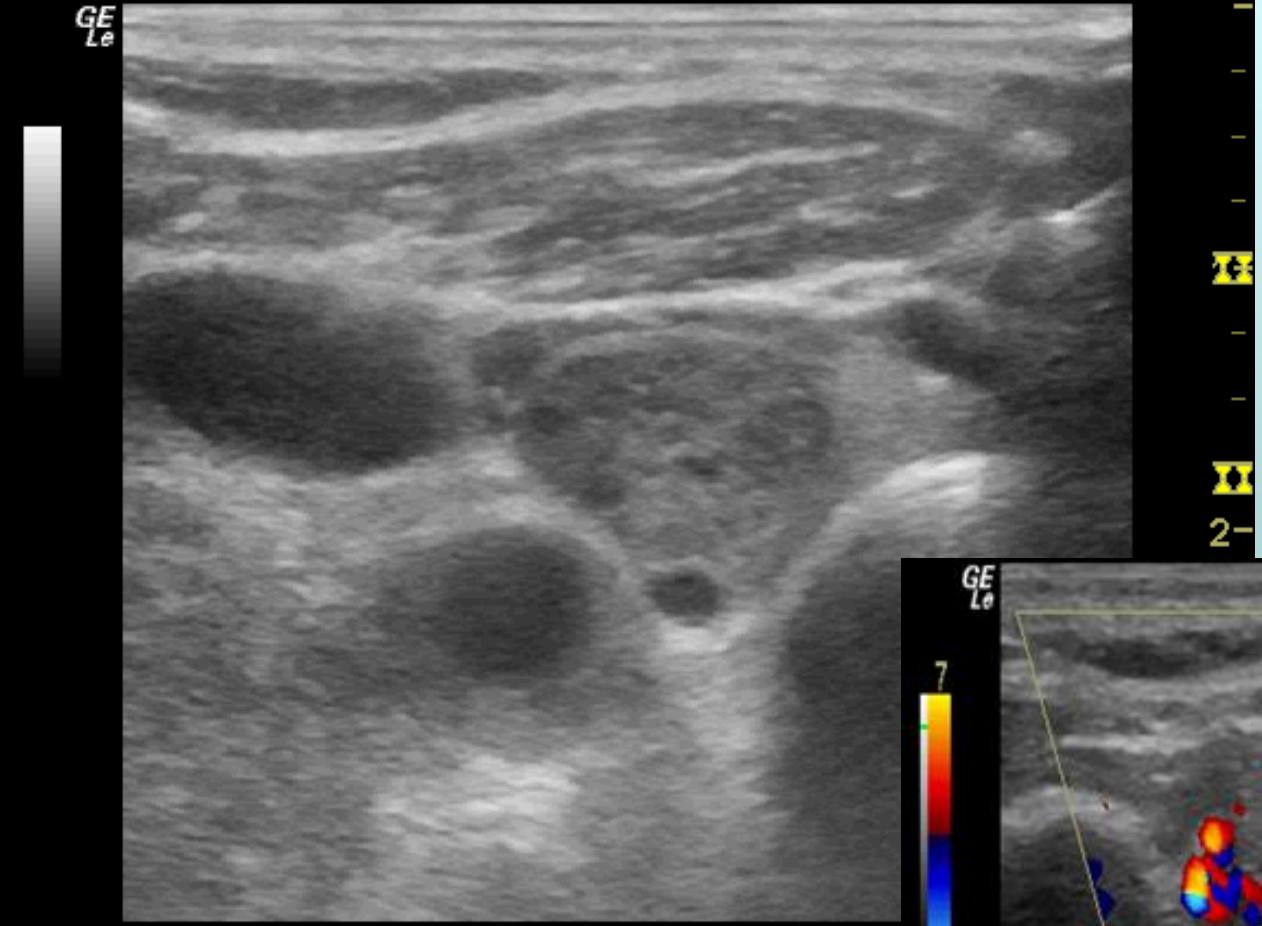
Has successfully completed a course in
Advanced Sonographic Procedures

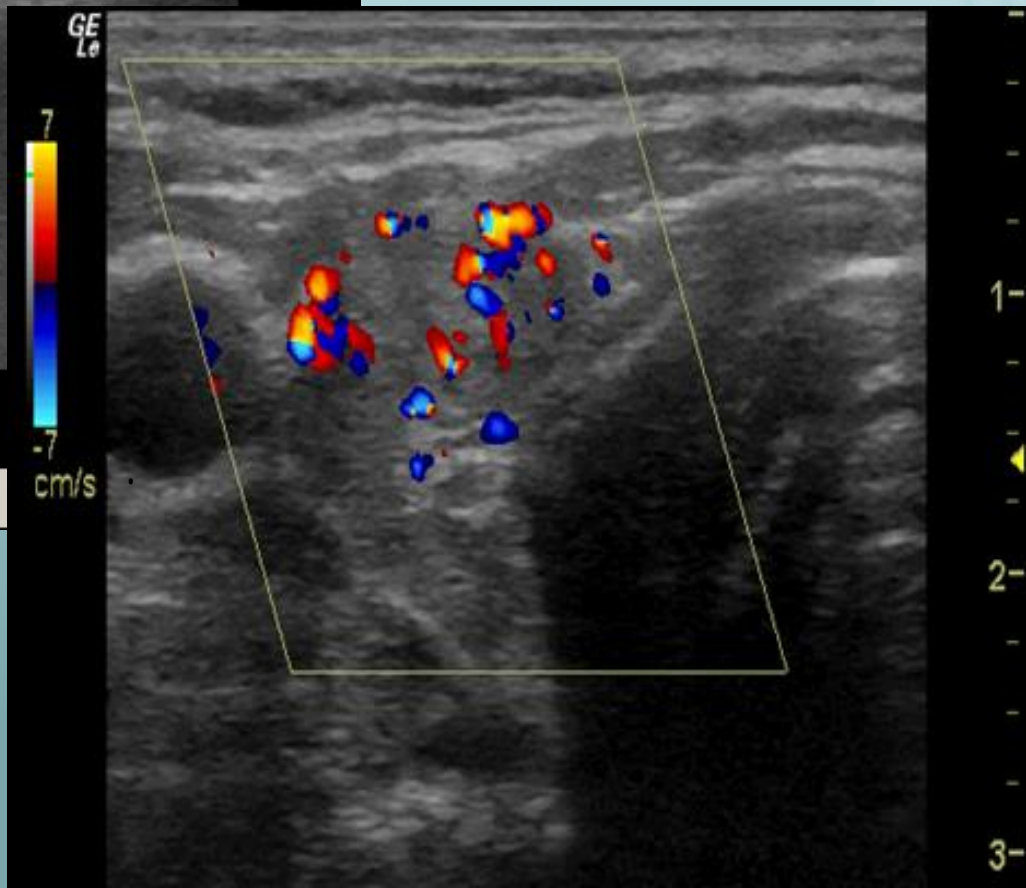
This 22nd day of January 2008

Coordinator, Health Careers

Program Director


ACCUVIX thyroid
XQ
Small Parts
#123 / 4.0cm MI 1.0
L5-12IM / RES TIs 0.0
2003-08-29
05:02:06 pm





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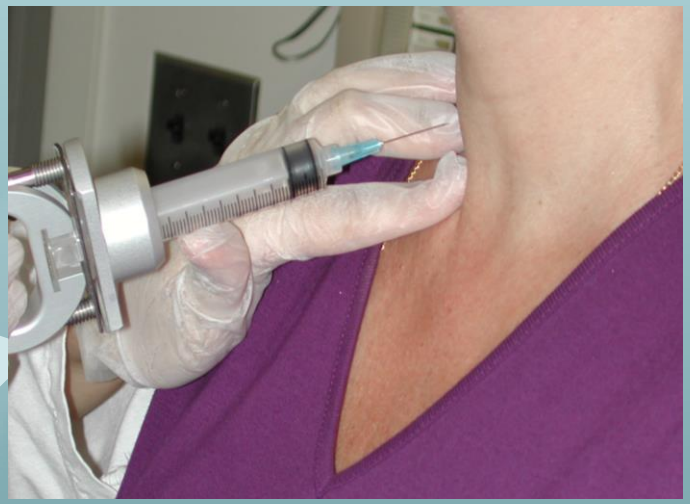
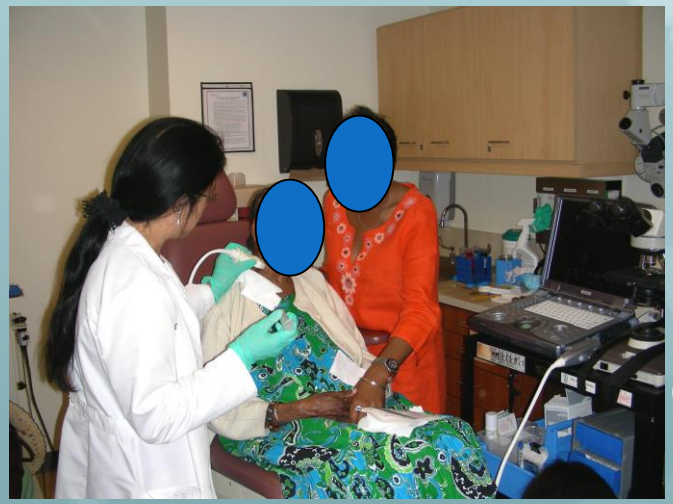
Head and Neck FNAs done by a Cytopathologist

100 PGFNA performed before July 2008



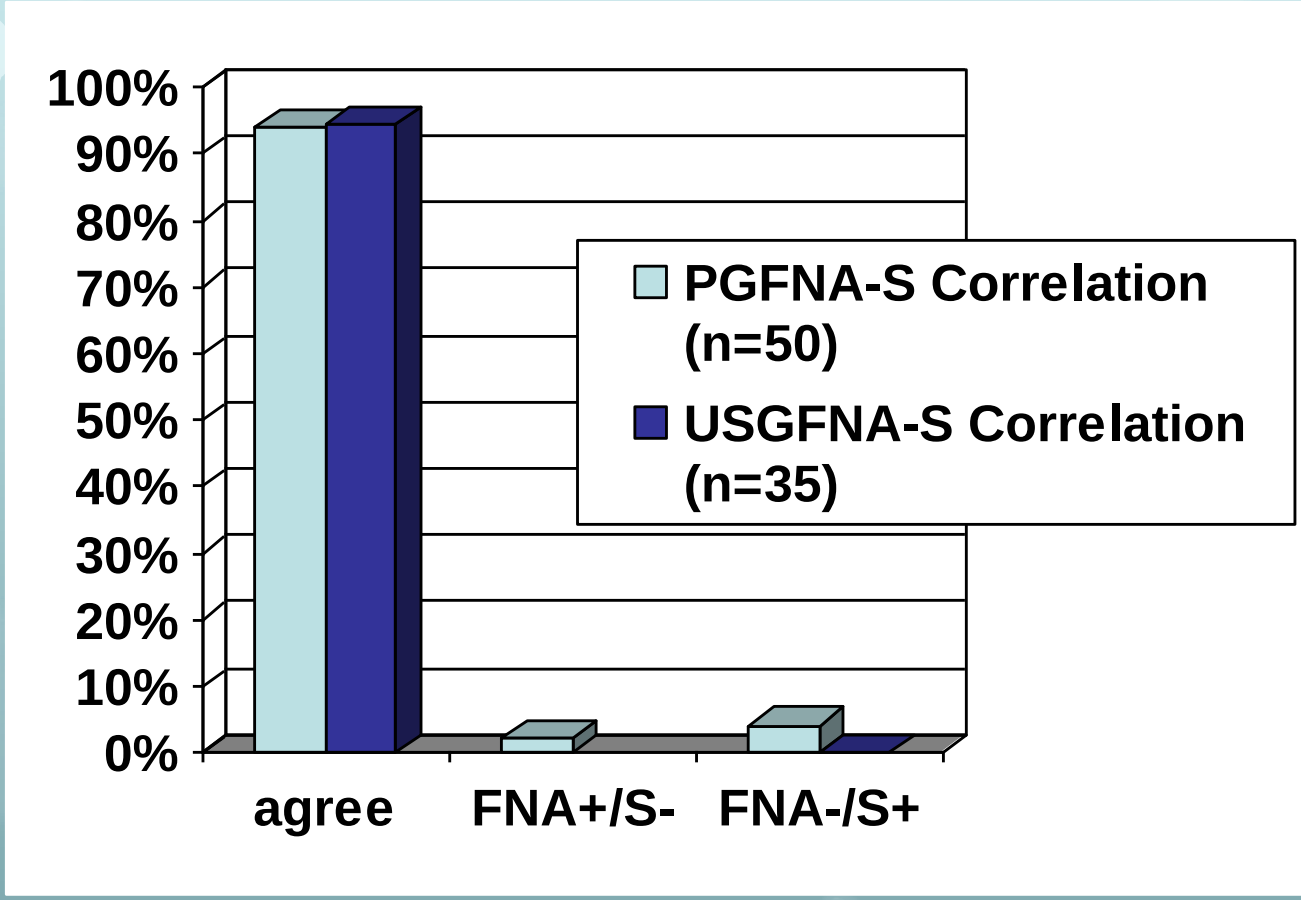
100 USGFNA performed between July 2008 and March 2009



Summary of FNA Diagnostic Rates

FNA type	Non-Path FNA (N=100)	Path-PGFNA (N=100)	Path-USGFNA (N=100)
Diagnostic rate (%)	24	83	86
Suspicious/ Atypical suggestive/ non-specific rate (%)	43	10	13
Non-diagnostic rate (%)	33	7	1

Correlation with Surgical



Mount Sinai

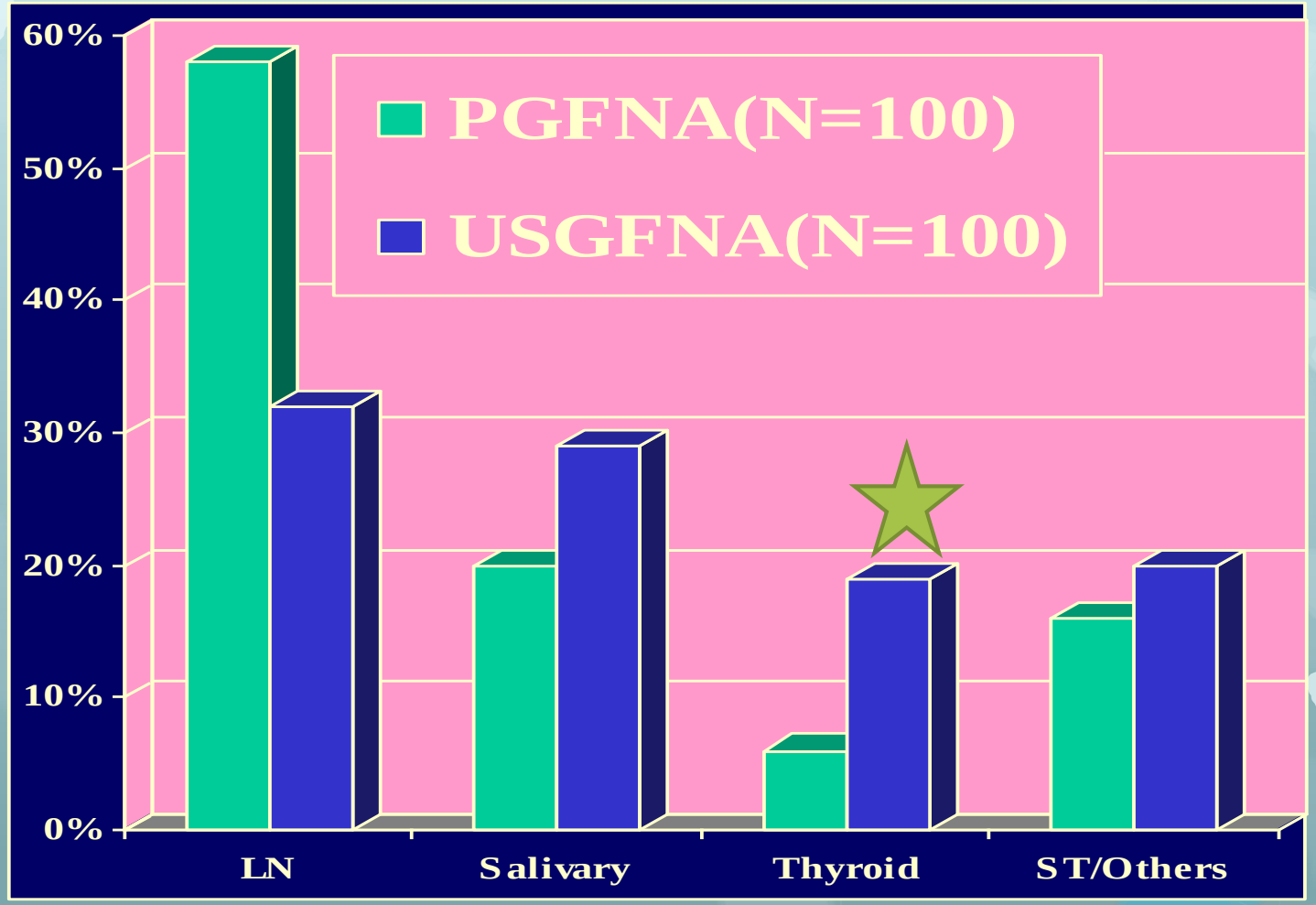
PGFNA vs. USGFNA with Surgical Follow-up

Total # of Cases (50)	**Positive Surgical**	**Negative Surgical**
Positive PGFNA	47 (94%) (TP)	1 (2%) (FP)
Negative PGFNA	2 (4%) (FN)	0 (TN)

Total # of Cases (35)	**Positive Surgical**	**Negative Surgical**
Positive USGFNA	28 (80%) (TP)	1(3%) (FP)
Negative USGFNA	0 (0%) (FN)	6 (17%) (TN)

Statistics	PGFNA	USGFNA
Sensitivity (%) (TP/TP+FN):	96	100
Specificity (%) (TN/TN+FP)	0	86
PPV (%) (TP/TP+FP)	98	97
NPV (%) (TN/TN+FN)	0	100

Case Distribution



Advantages of US-Guidance

Non- palpable lesions

Small lesion (0.5cm)

Target solid and cystic areas

Specific diagnosis (lipoma)

Significantly improved Specificity and NPV

The evidence indicates:

With US-guidance, FNA performed by a Cytopathologist may achieve not only superior Sensitivity and PPV, but also excellent Specificity and NPV.

All Thyroid FNA biopsies should be performed under ultrasound guidance.

Ultrasound Features

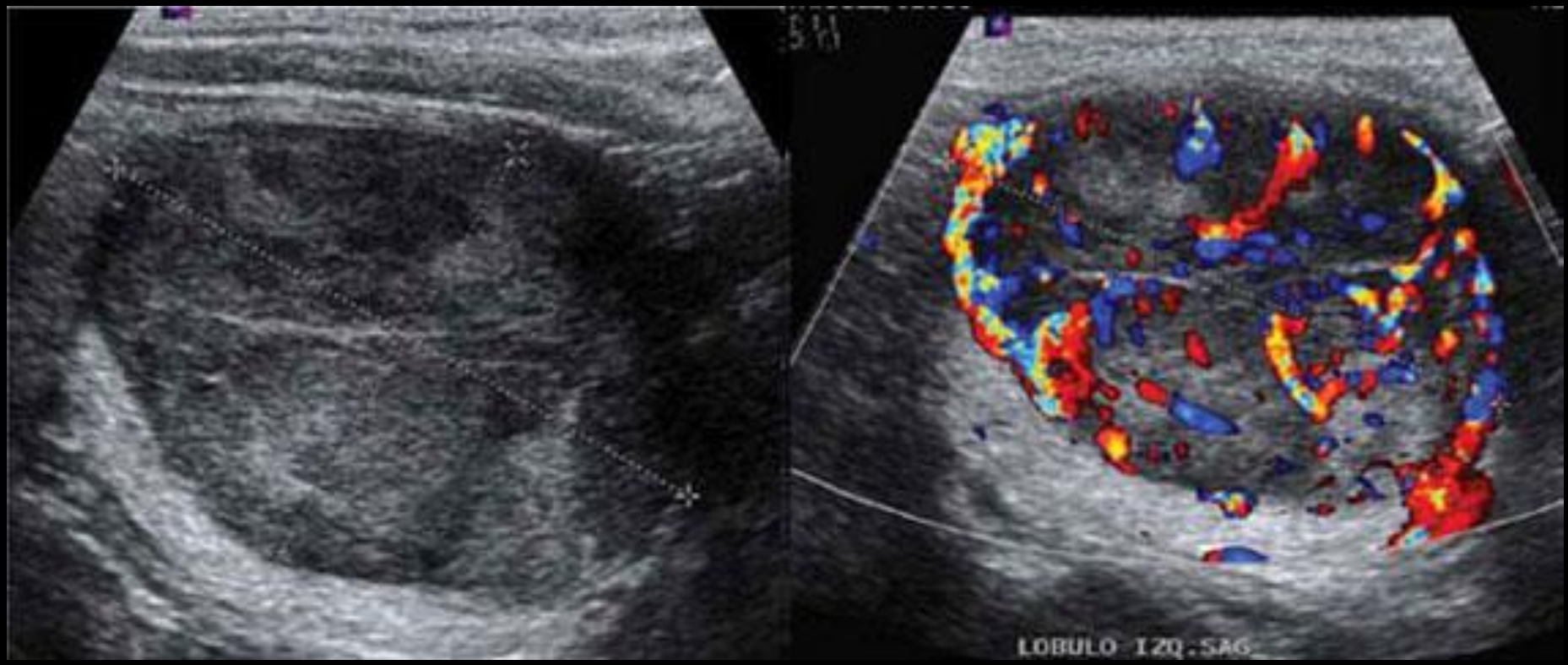
High Risk

- Hypoechoic (26%)
- Increased central chaotic vascularity
- Fine punctate/psammoma bodies Microcalcifications
- Incomplete halo
- Irregular borders
- Taller than wide
- Suspicious lymph nodes

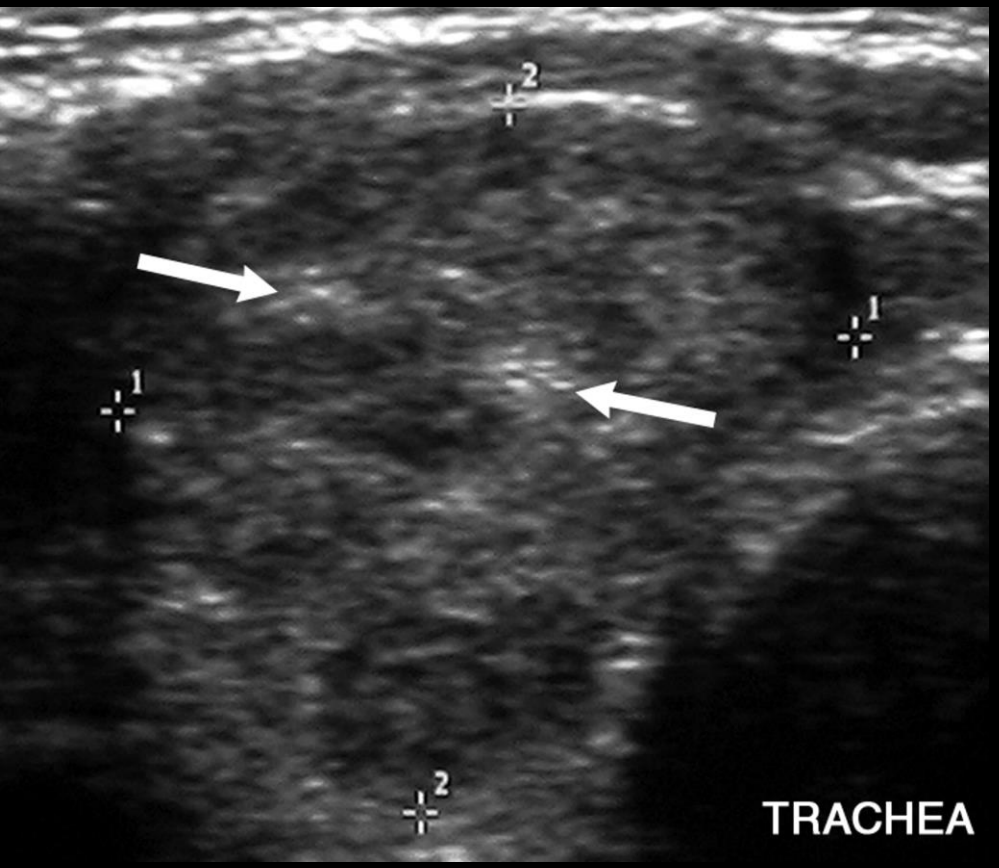
Low Risk

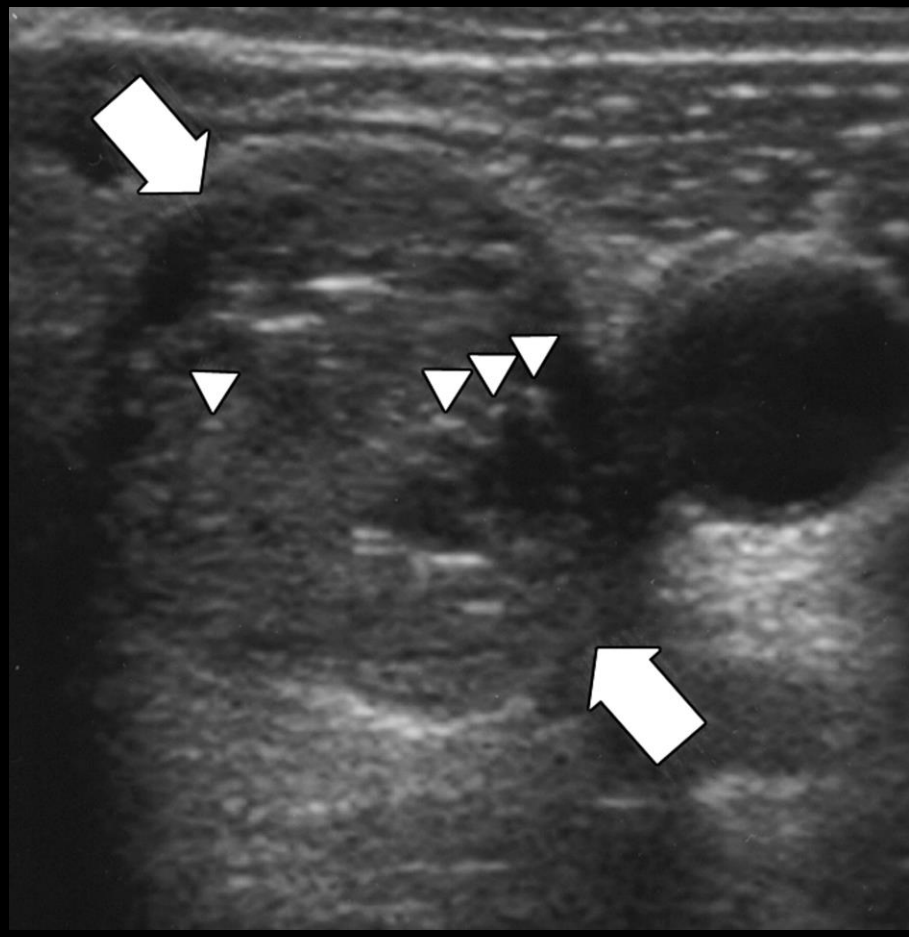
- Hyperechoic (4%)
- Absence/perinodular vascularity
- Large, coarse, dysmorphic or curvilinear calcifications
- Complete Halo
- Regular borders
- Flat lesion
- Comet-tail (cystic lesion)

Central Vascularity

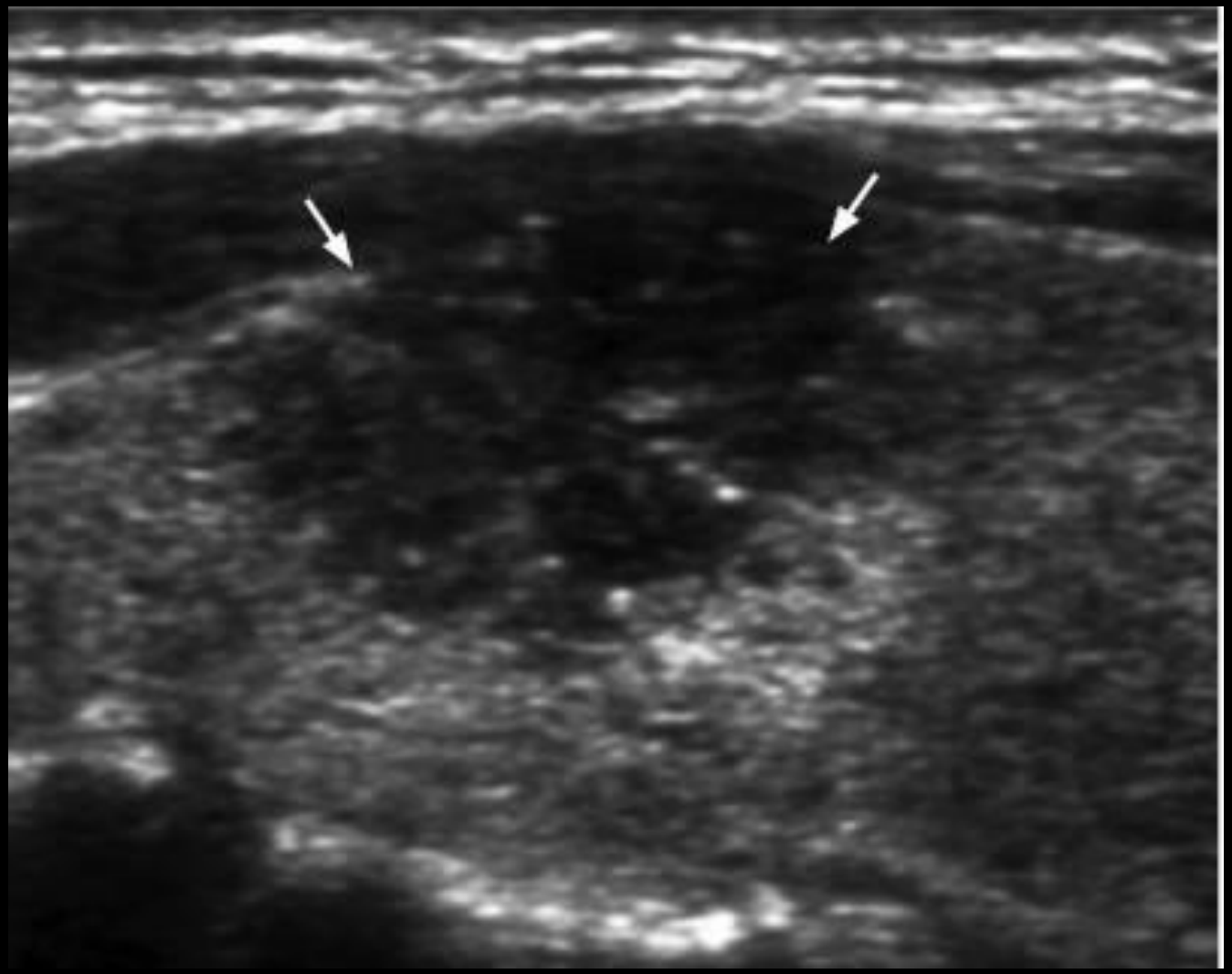


Microcalcifications

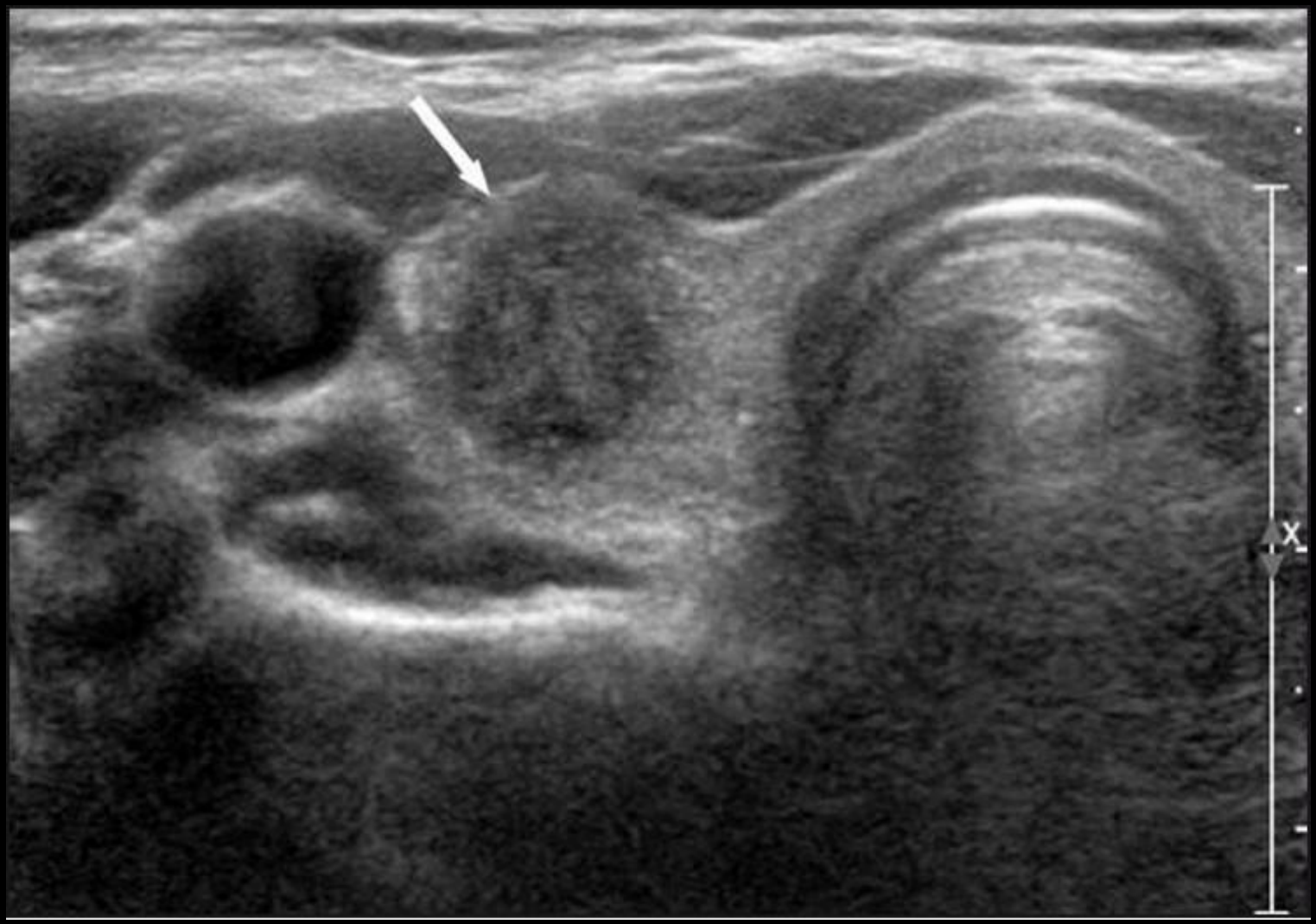




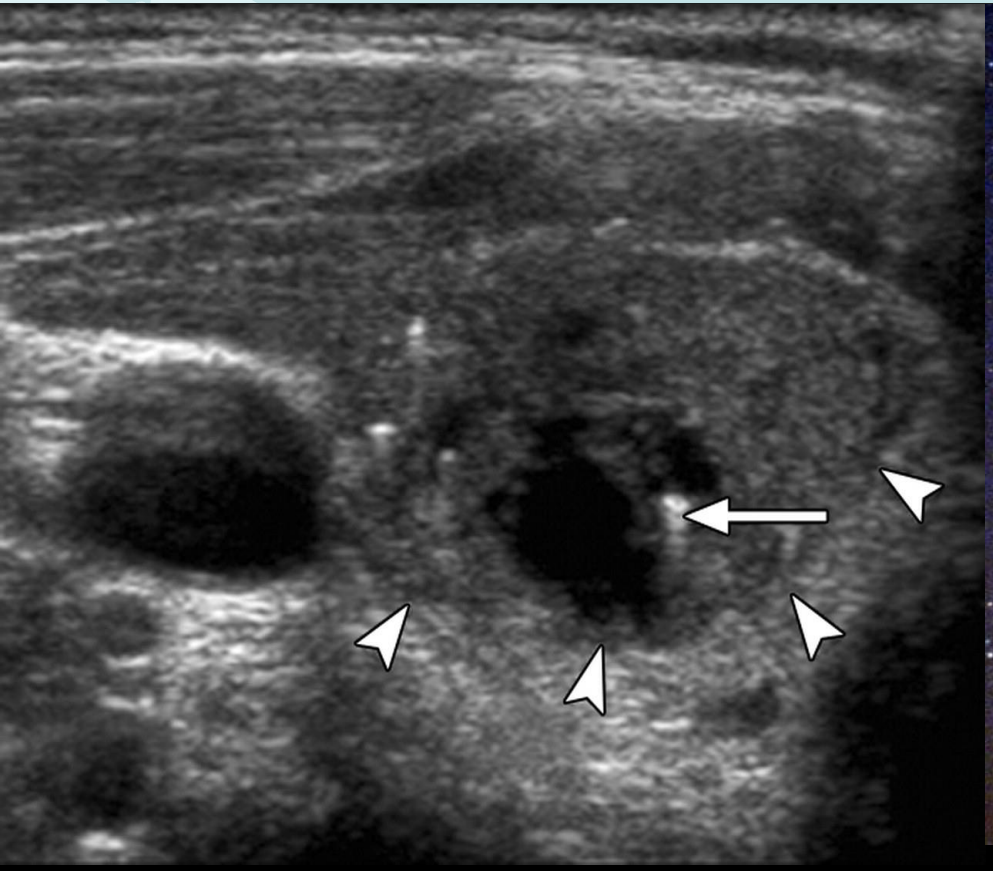
Irregular Borders



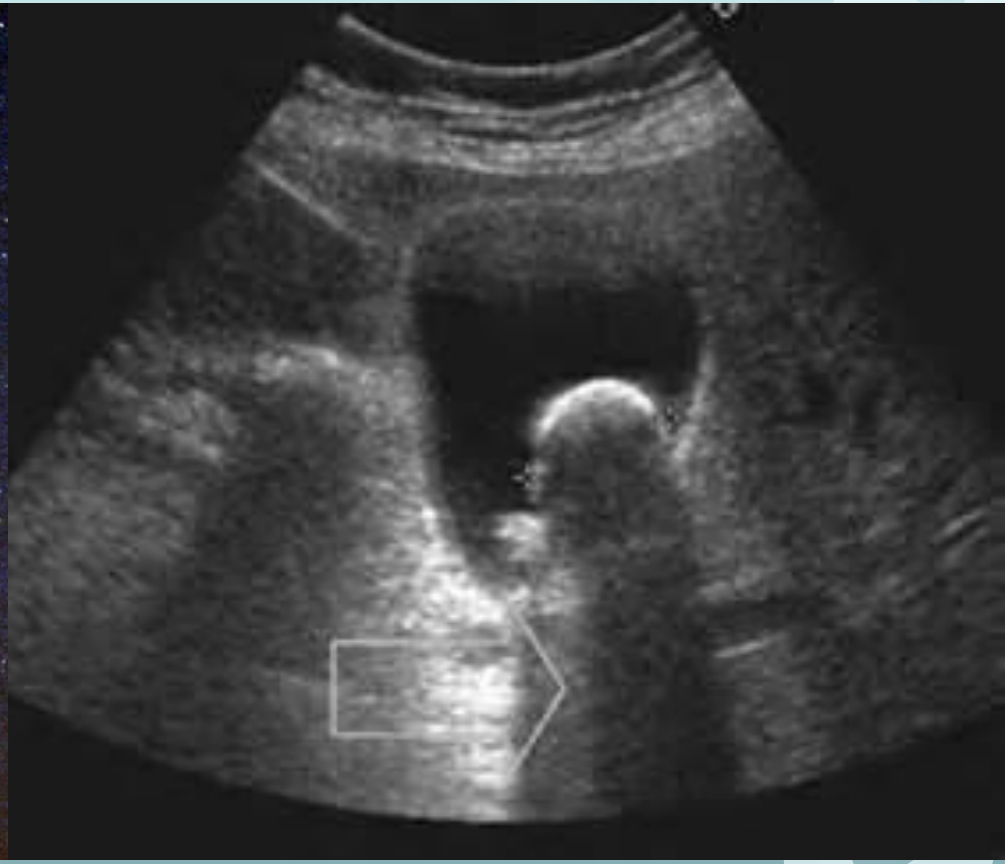
Taller Than Wide



Comet-tail Artifact



Comet-tail vs. Attenuation



The Bethesda System for Reporting Thyroid Cytopathology

I. Nondiagnostic or Unsatisfactory

- Cyst fluid only; Virtually acellular specimen; Other (obscuring blood, clotting artifact, etc))

II. Benign

- Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc)
- Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context
- Consistent with granulomatous (subacute) thyroiditis

III. Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance

IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm

- Specify if Hürthle cell (oncocytic) type

V. Suspicious for Malignancy

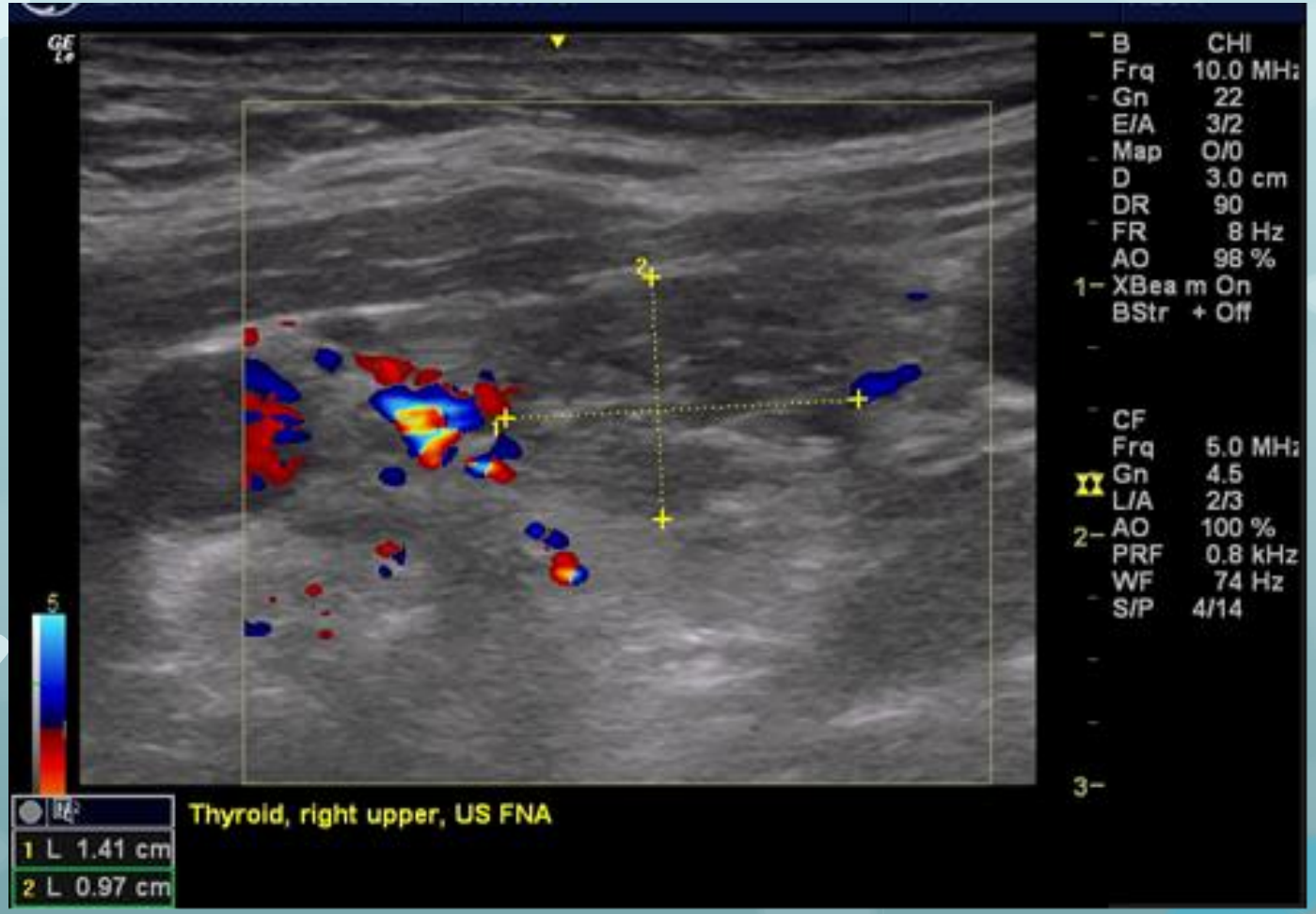
- Suspicious for papillary carcinoma, medullary carcinoma, metastatic carcinoma, lymphoma, etc.

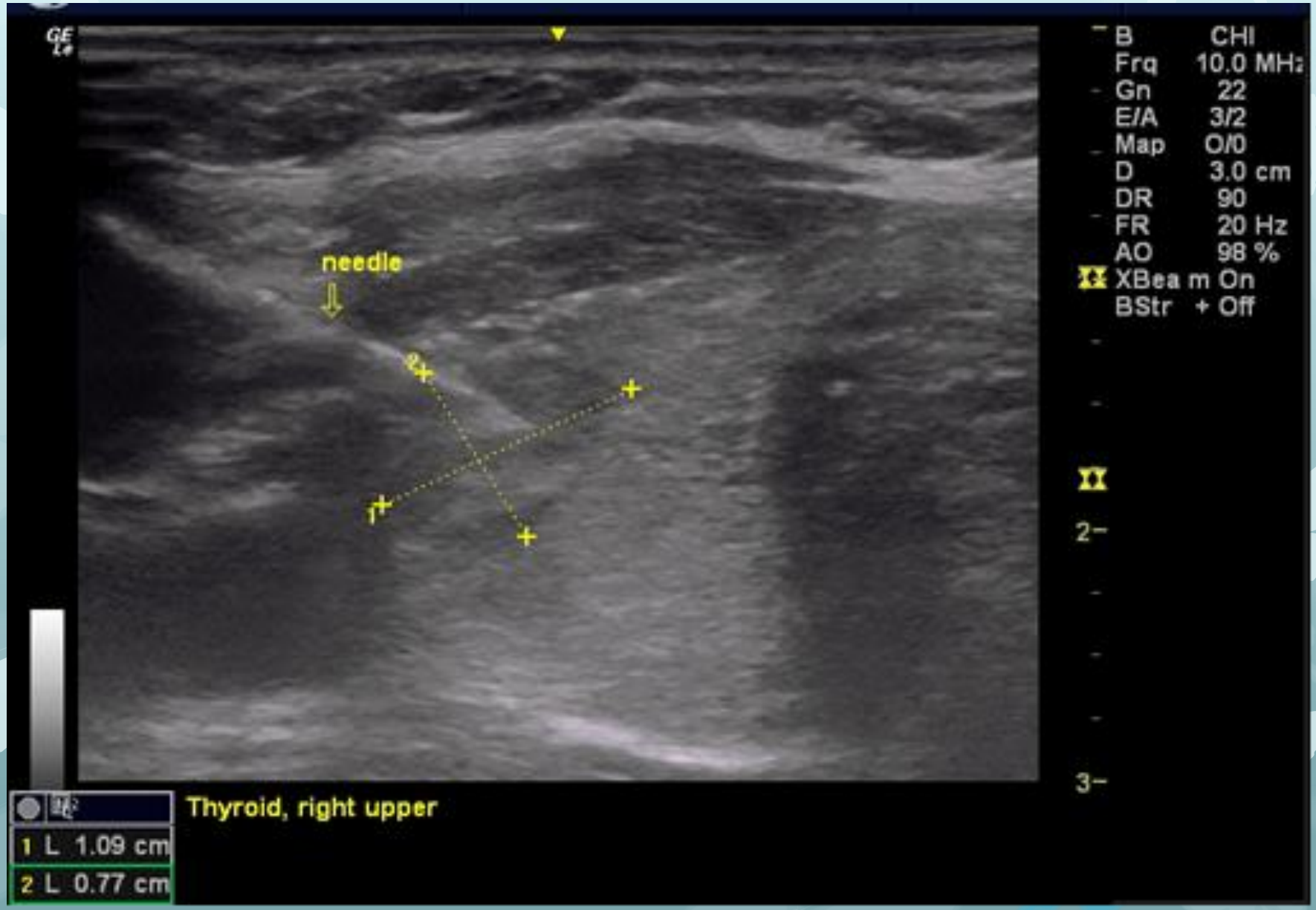
VI. Malignant

- Papillary thyroid carcinoma, Poorly differentiated carcinoma, Medullary thyroid carcinoma, Undifferentiated (anaplastic) carcinoma, Squamous cell carcinoma, Carcinoma with mixed features (specify), Metastatic carcinoma, Non-Hodgkin lymphoma, etc.

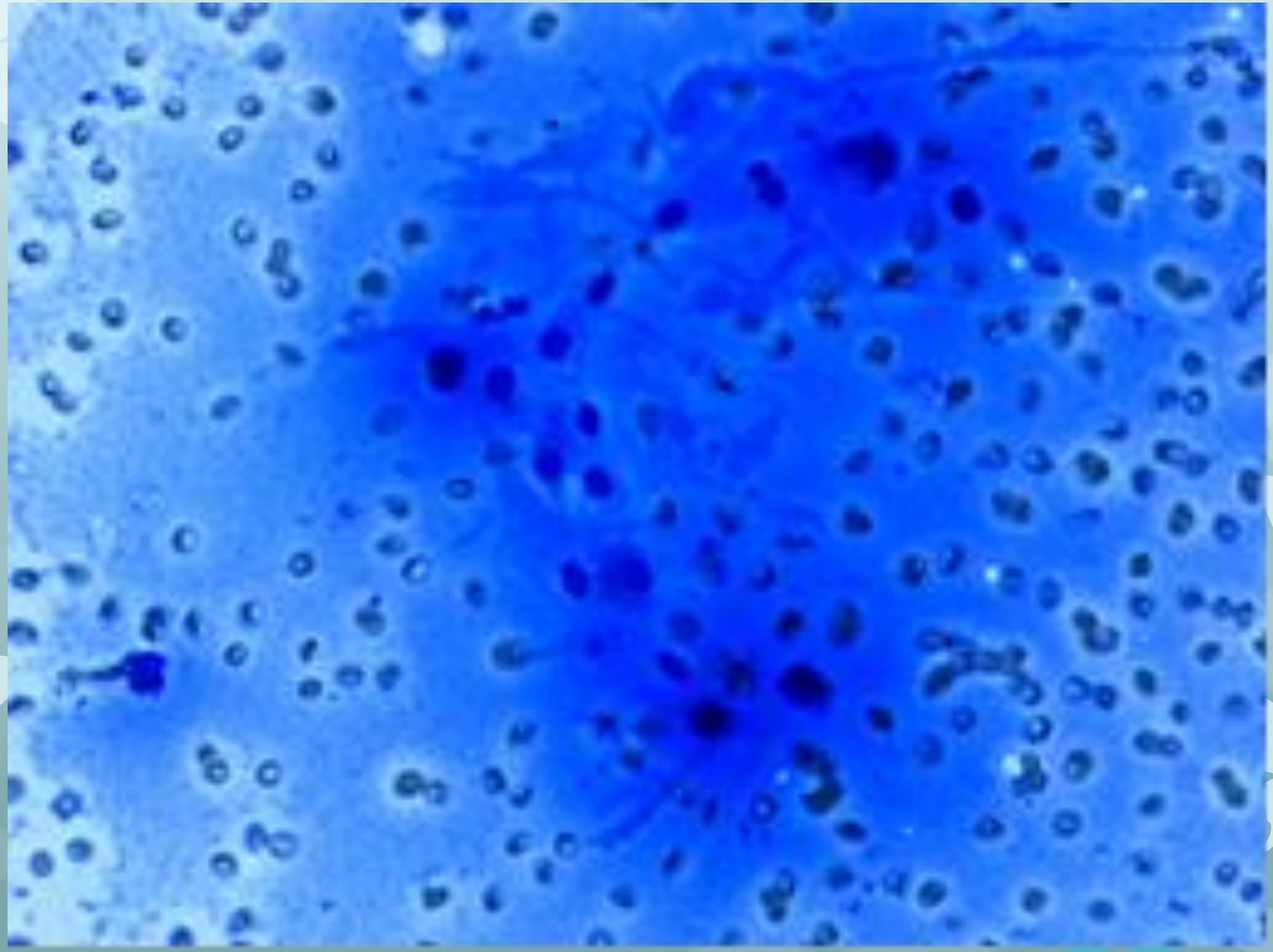
Case 1

Middle-aged woman with incidental thyroid nodule




needle
B CHI
Frq 10.0 MHz
Gn 22
E/A 3/2
Map O/0
D 3.0 cm
DR 90
FR 20 Hz
AO 98 %
XBeam On
BStr + Off
2
3
Thyroid, right upper
1 L 1.09 cm
2 L 0.77 cm

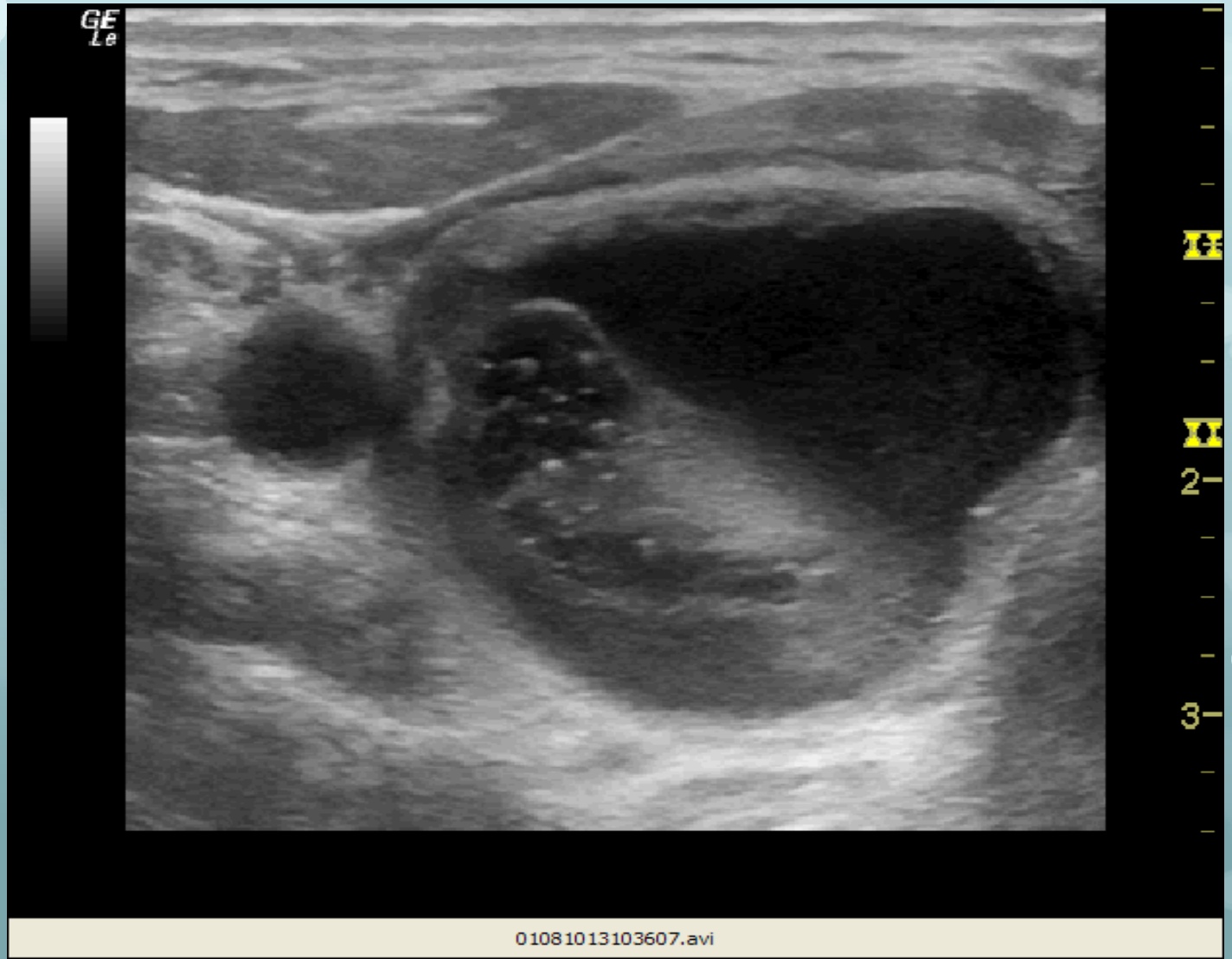
On site evaluation

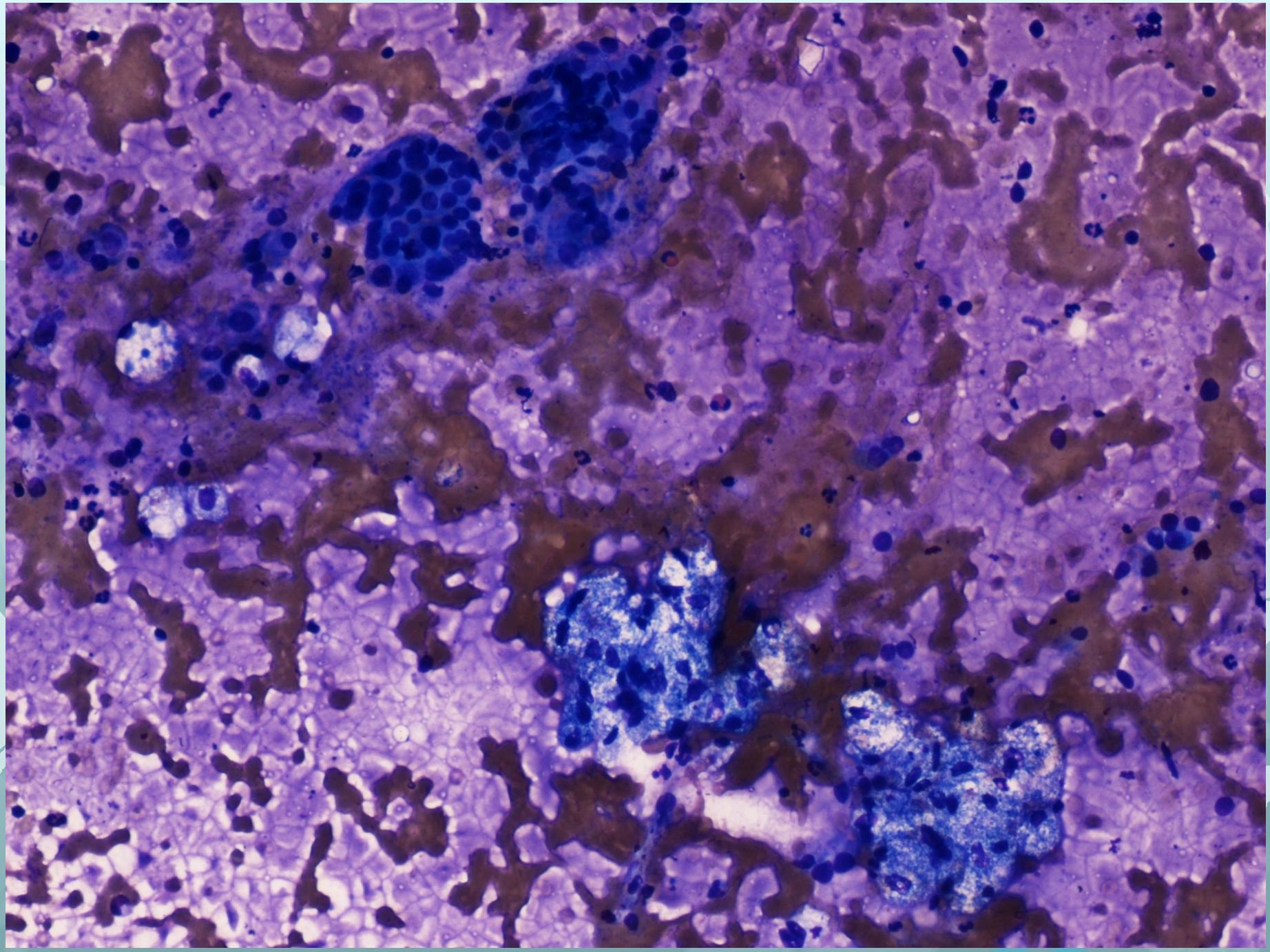


Final Cytological Diagnosis for Case 1

Benign follicular nodule
(Bethesda Category II)

Case 2
71 female with Rt Thyroid Nodule





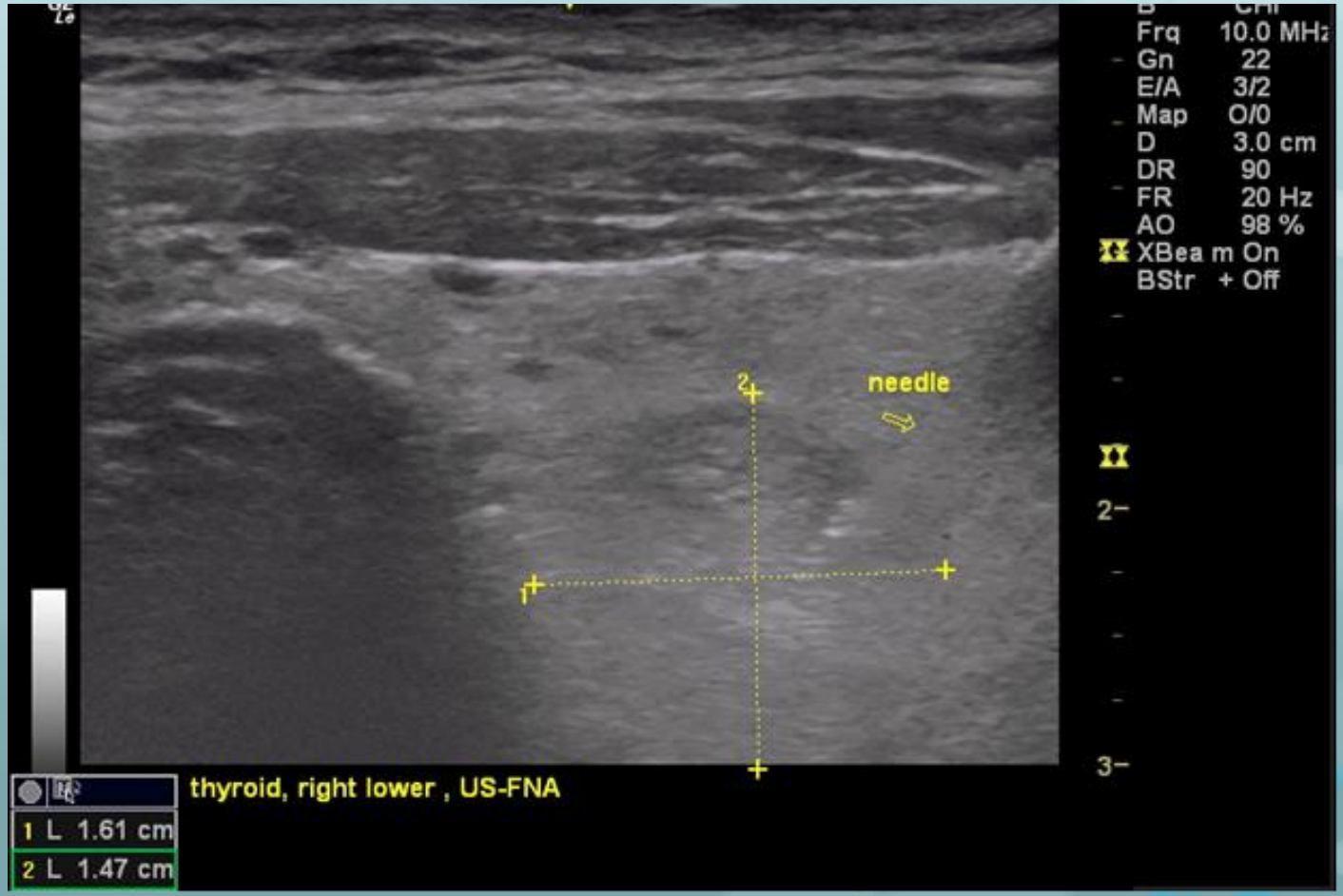
Final Cytologic Diagnosis for Case 2

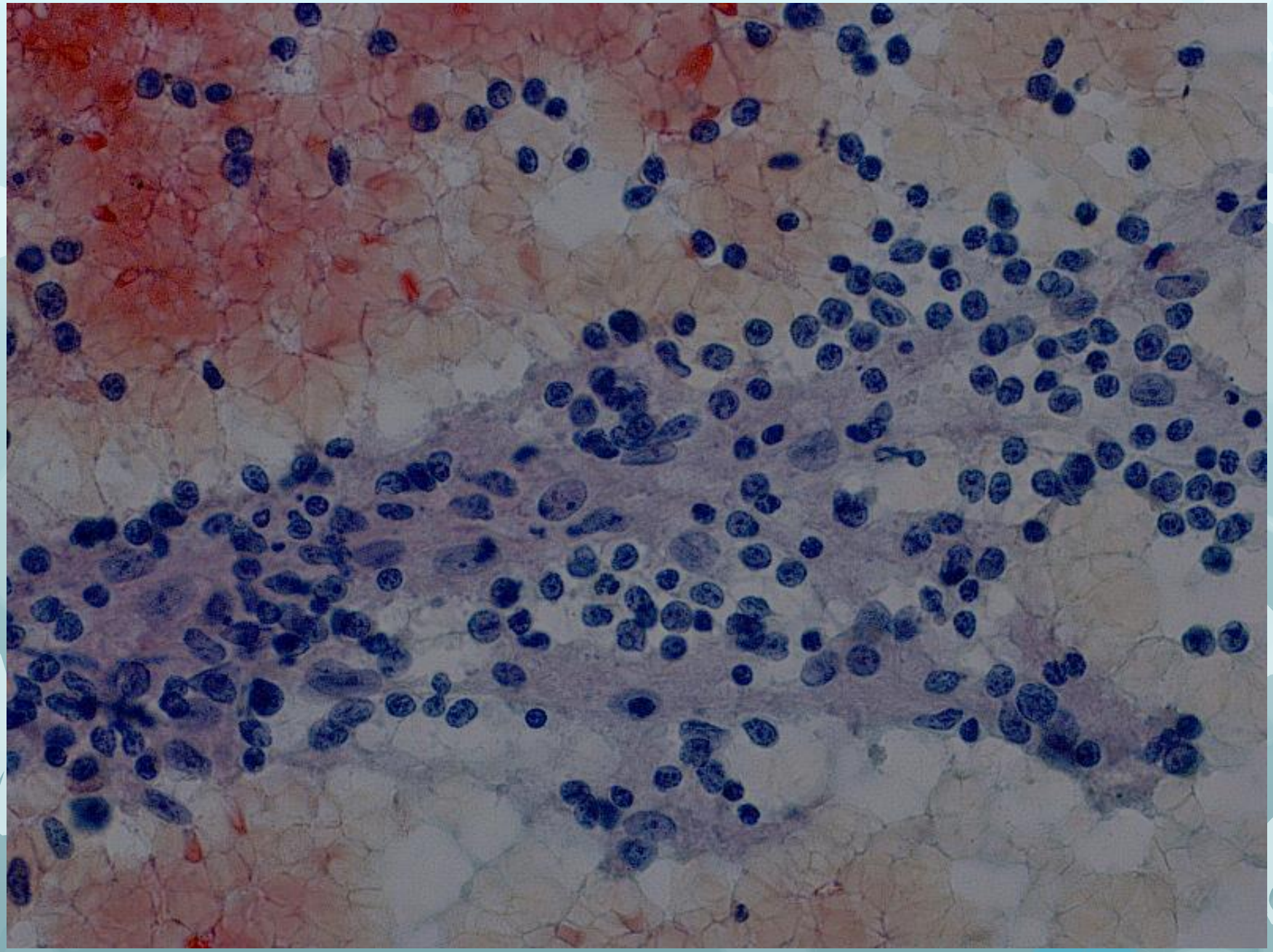
Benign follicular nodule

with hemorrhagic cystic changes

(Bethesda Category II)

Case 3
Mid age women with ill-defined thyroid nodules



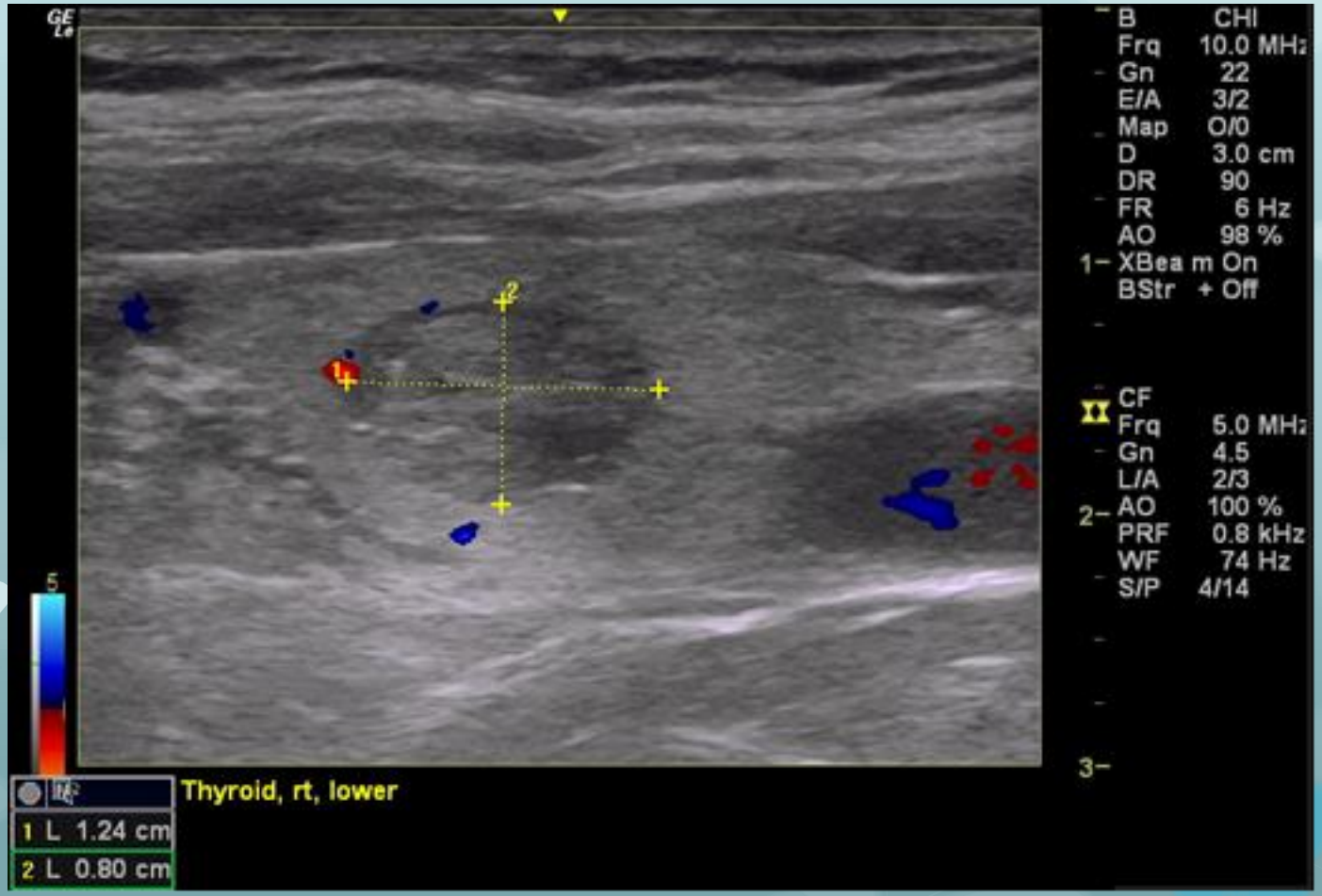


Final Cytological diagnosis for Case 3

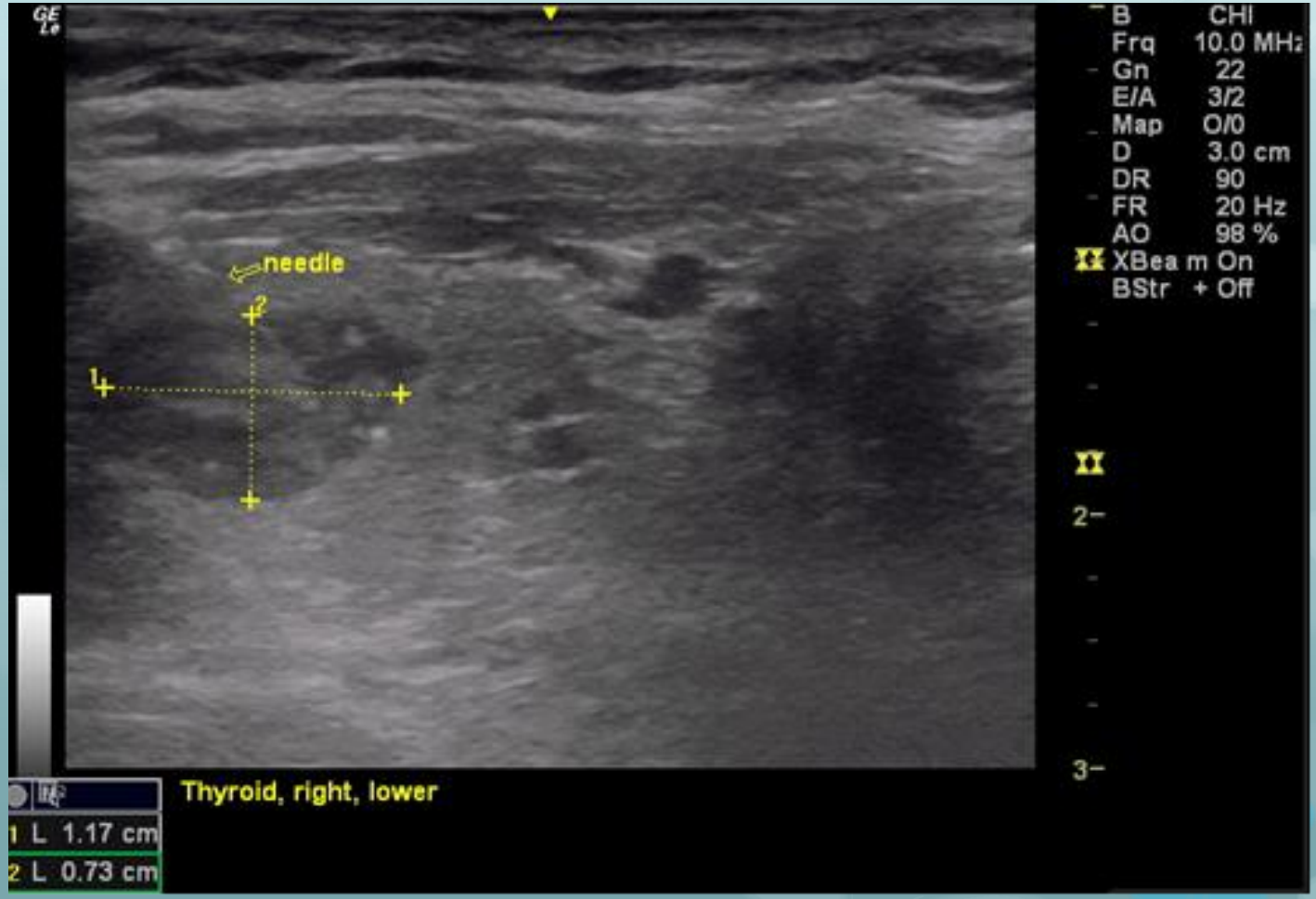
Benign, suggestive of lymphocytic thyroiditis in a proper clinical context

(Bethesda category II)

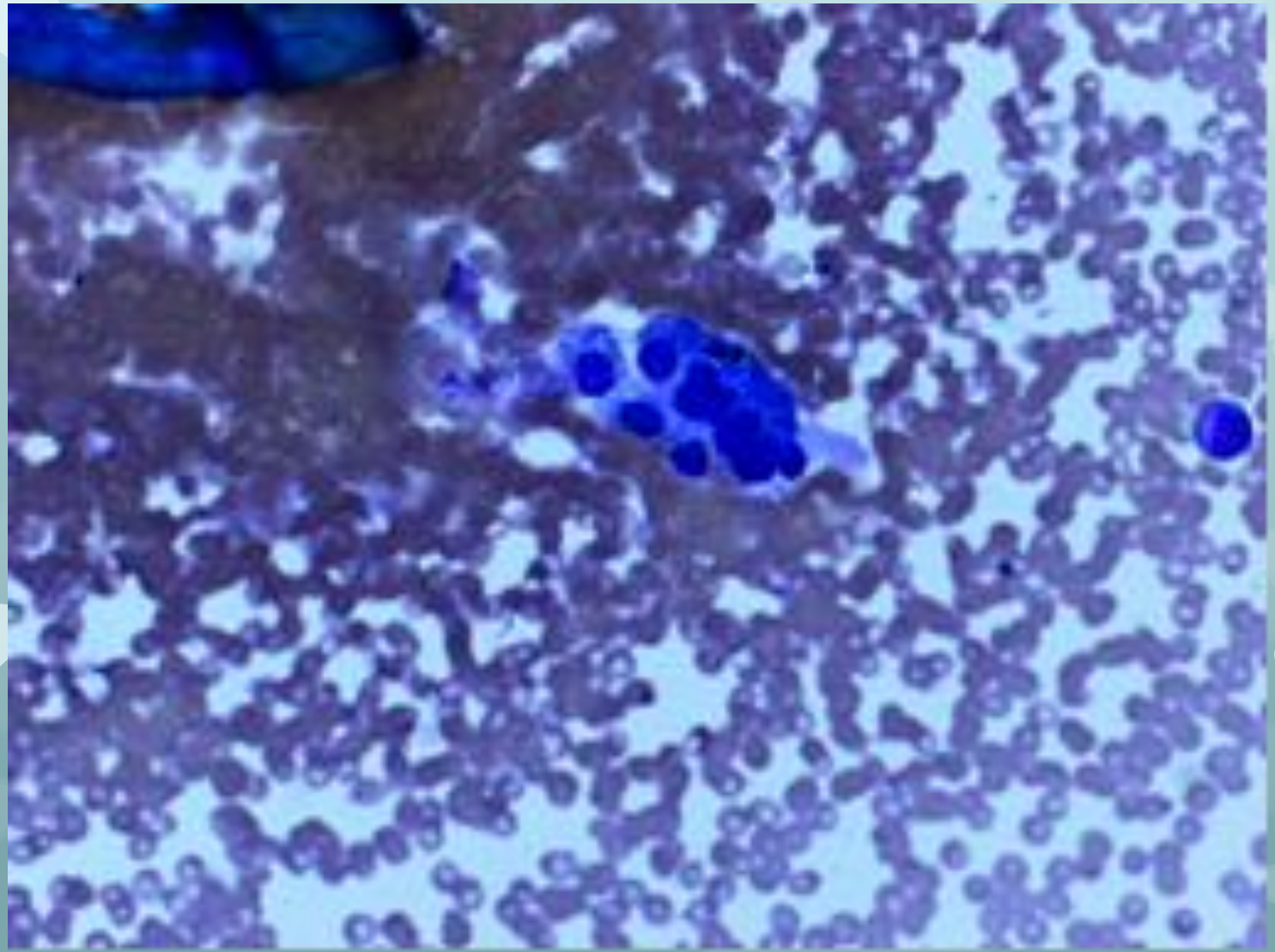
Case 4
Elderly male with incidental thyroid nodule

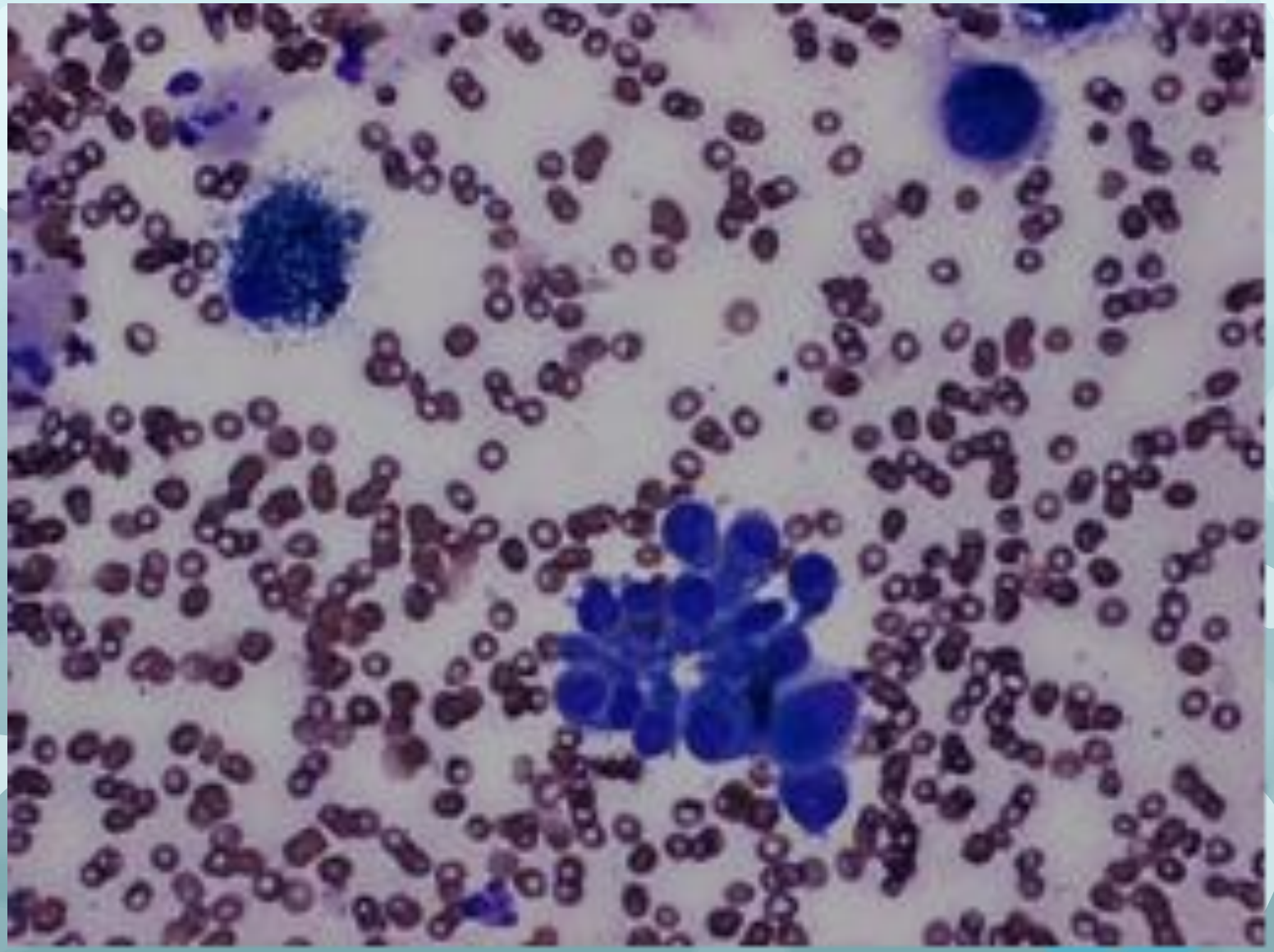


US-FNA of Case 4

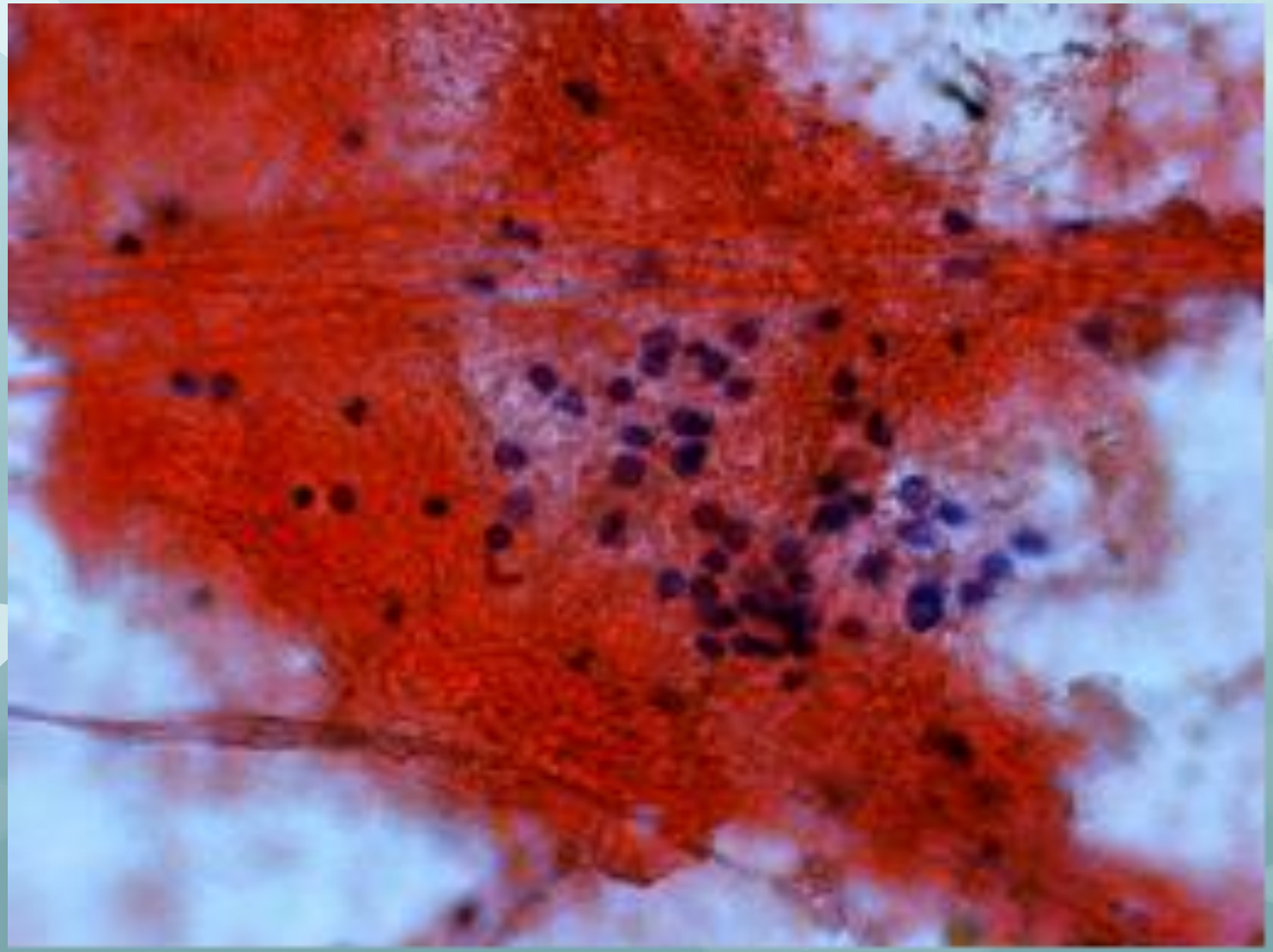


On-site evaluation





Pap

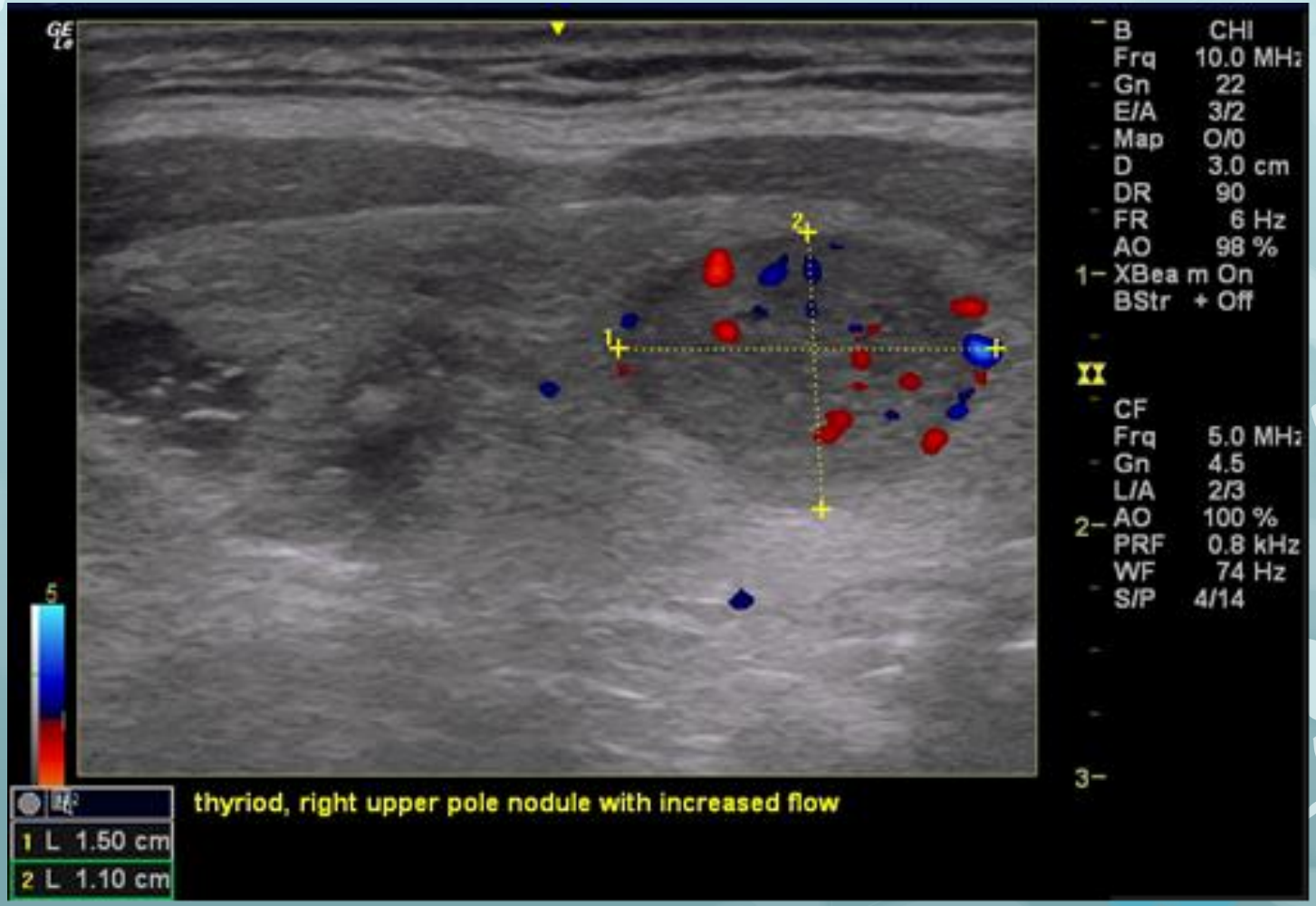


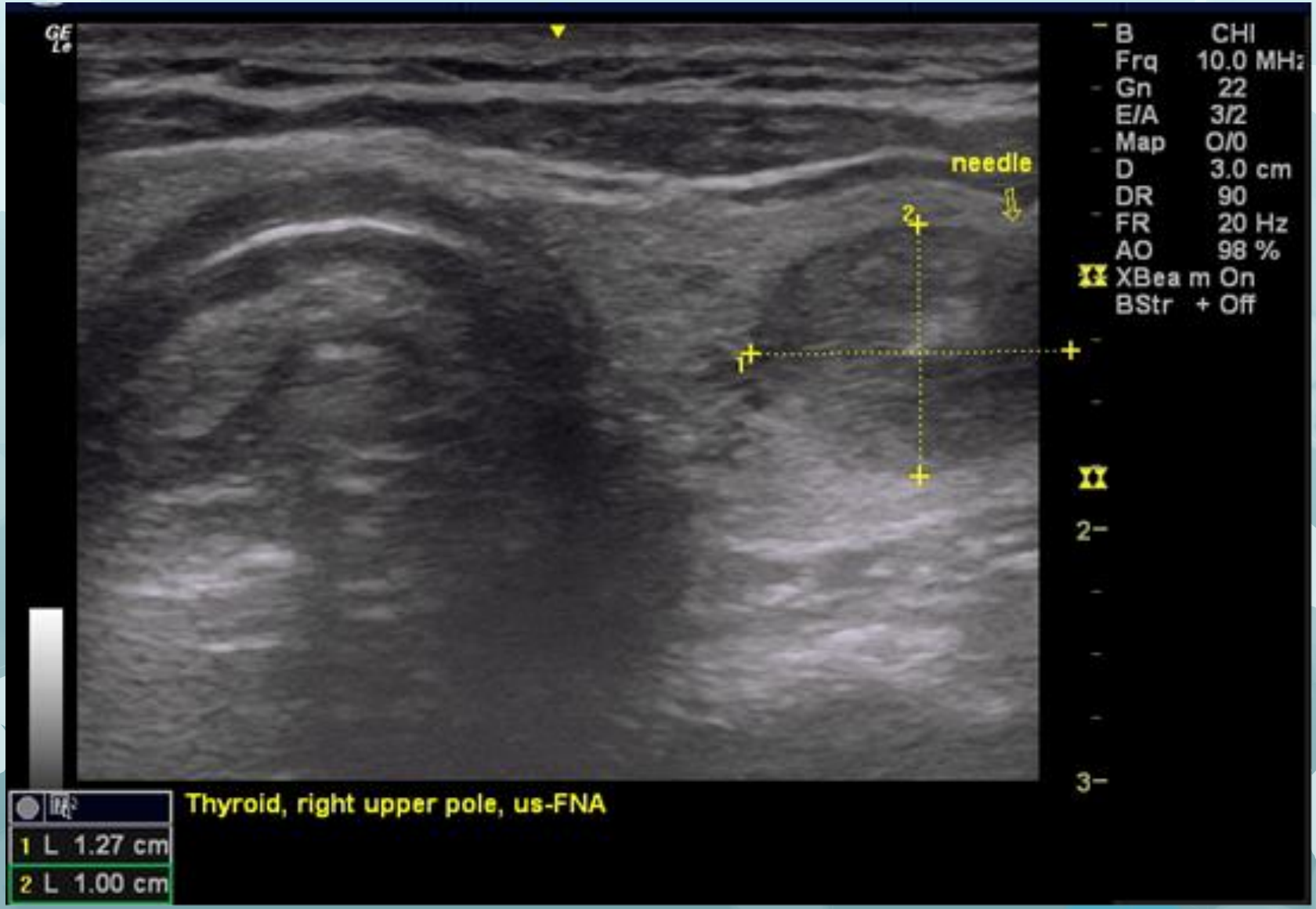
Final Cytologic Diagnosis for Case 4

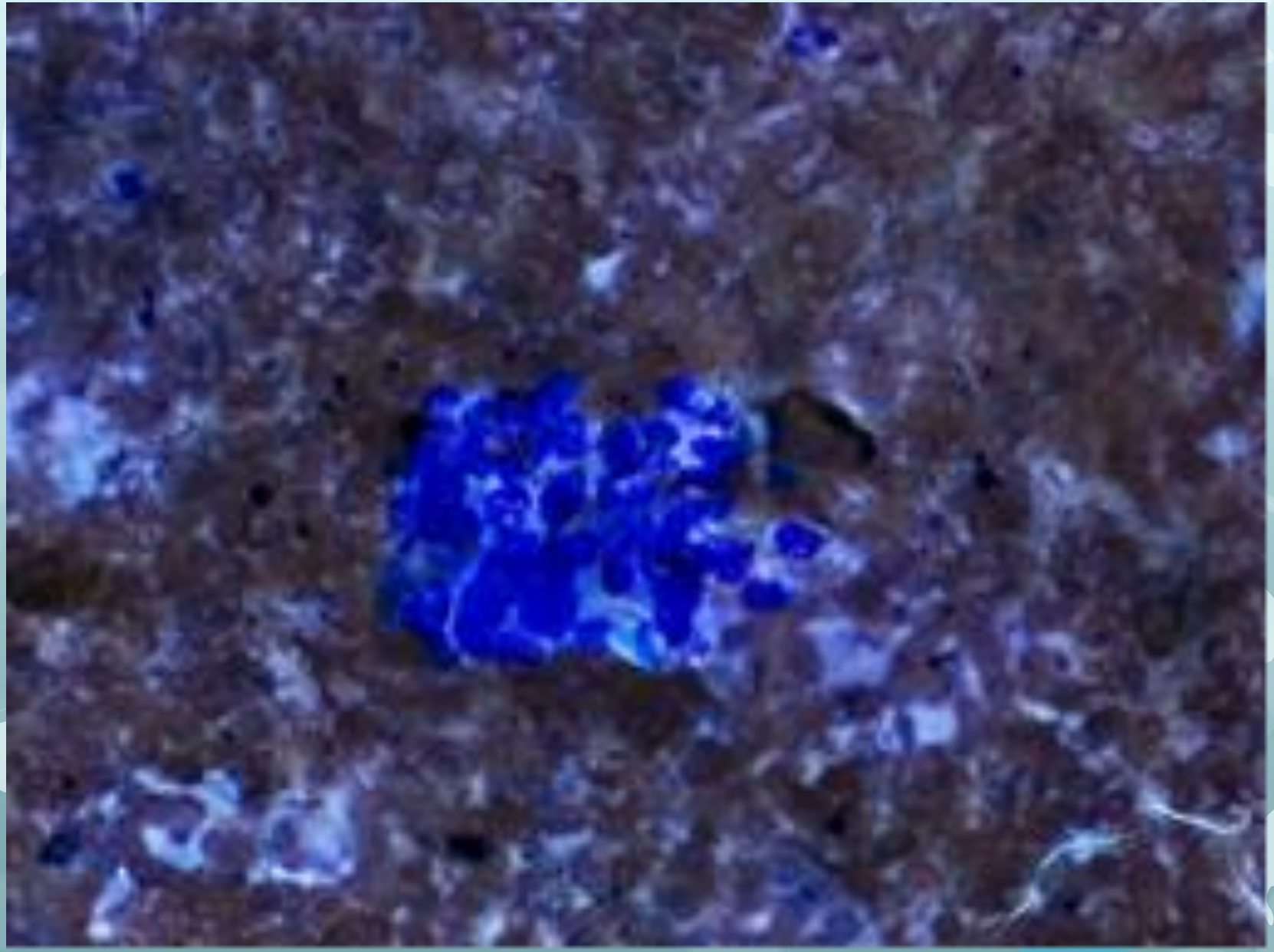
Focally Atypical Follicular lesion with peripherally increased blood flow

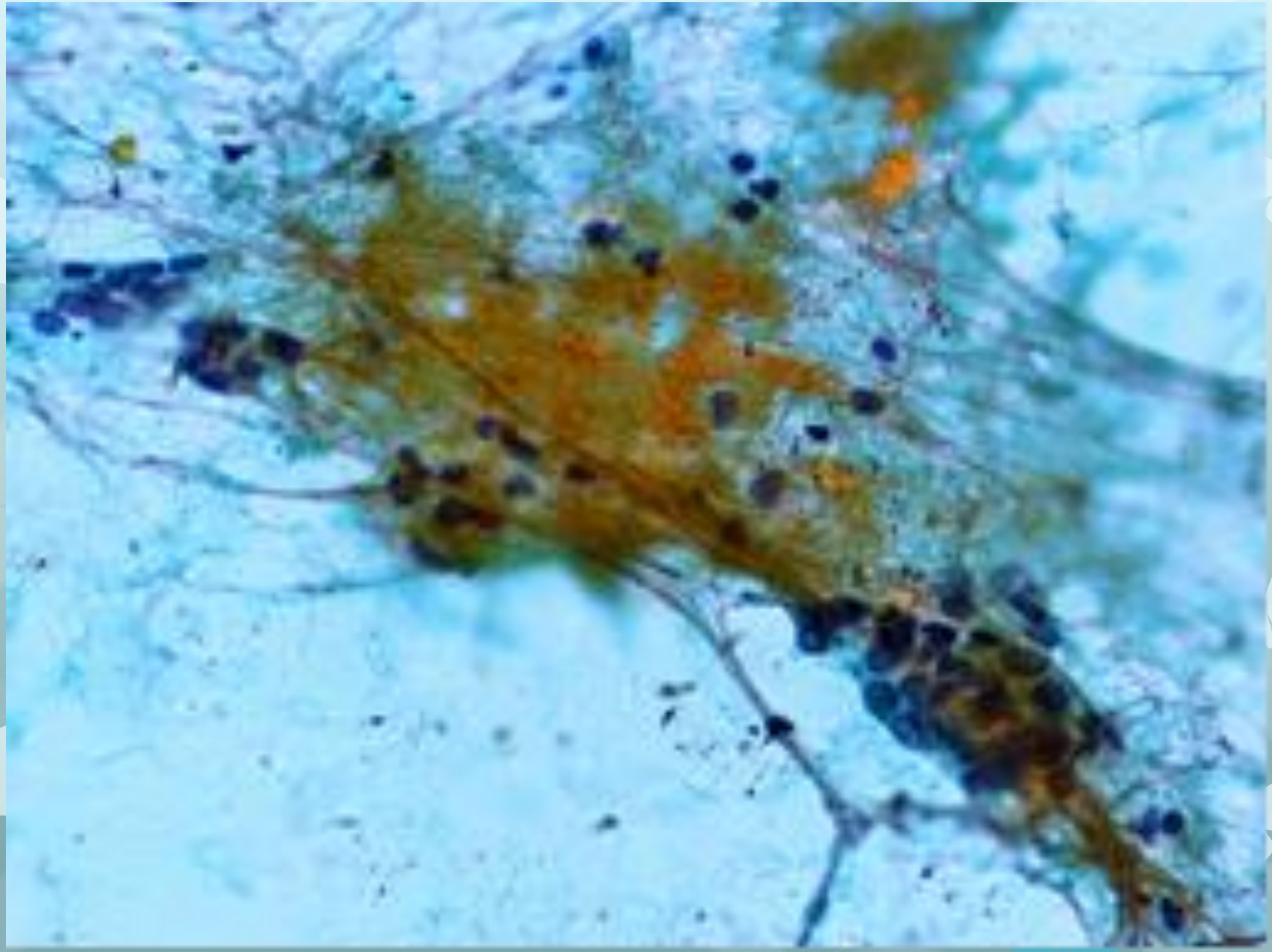
(Bethesda category III)

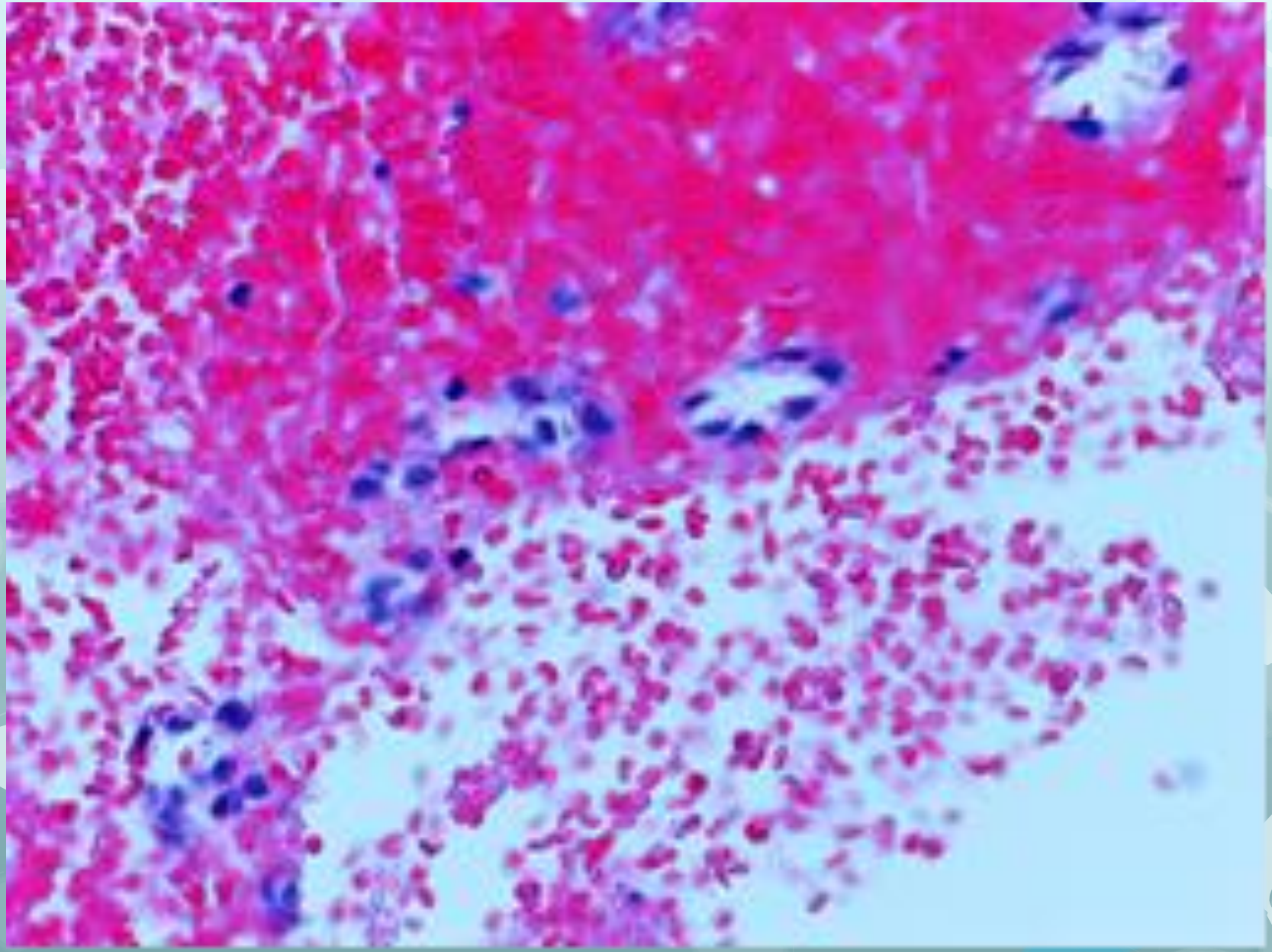
Case 5
Middle-aged woman with hypervascular thyroid nodule




needle
B CHI
Frq 10.0 MHz
Gn 22
E/A 3/2
Map O/0
D 3.0 cm
DR 90
FR 20 Hz
AO 98 %
XBeam On
BStr + Off
Thyroid, right upper pole, us-FNA
1 L 1.27 cm
2 L 1.00 cm



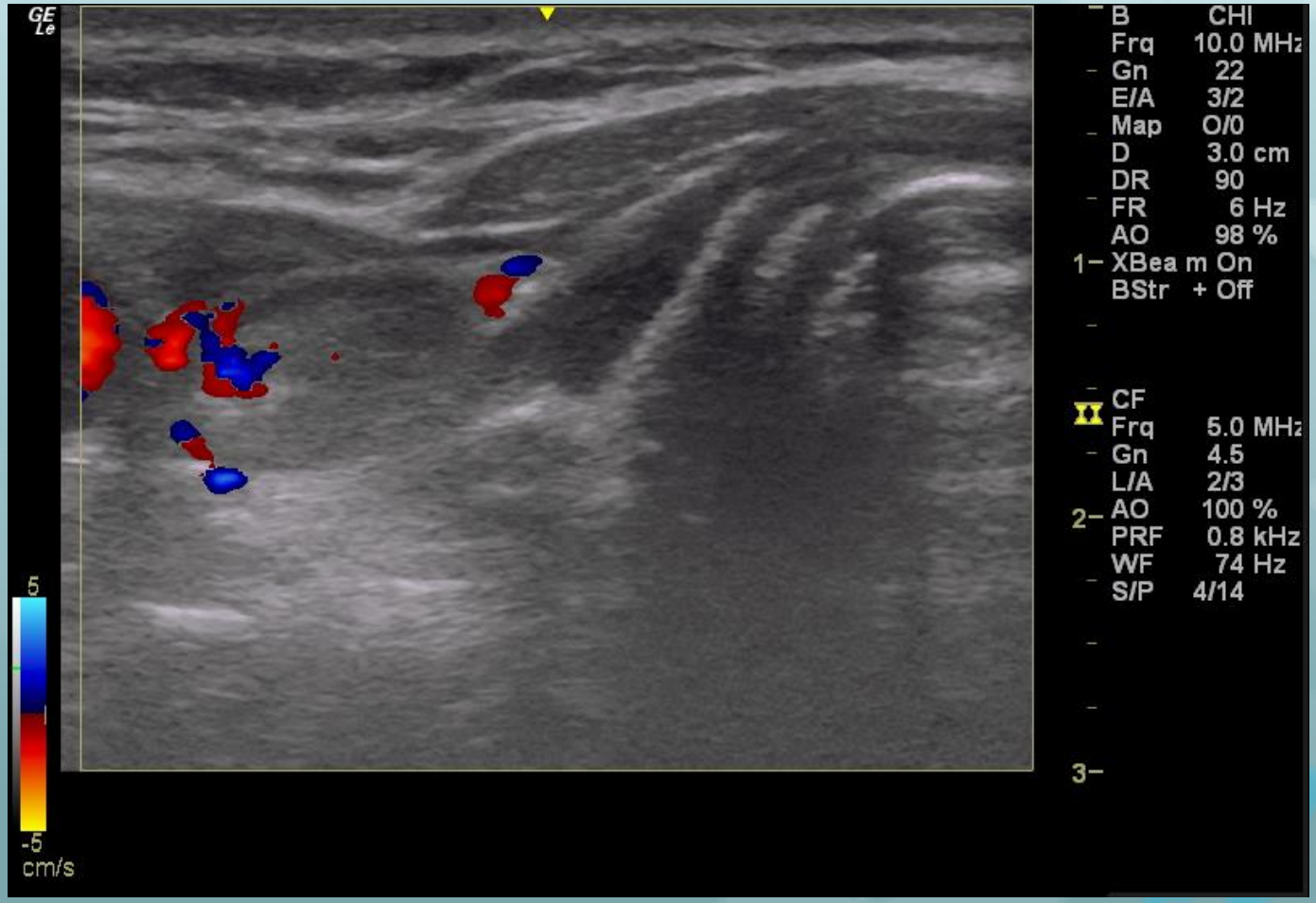


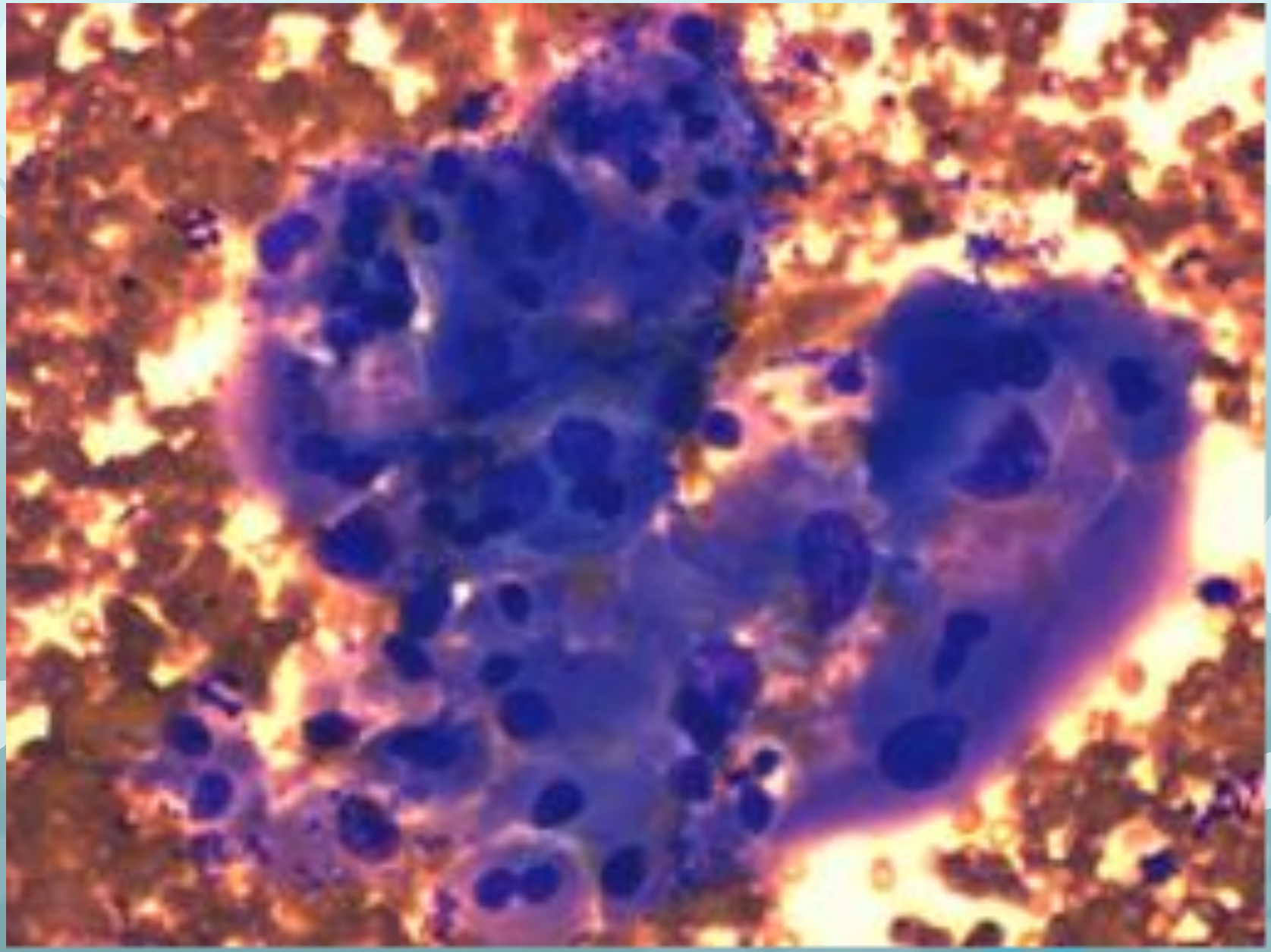


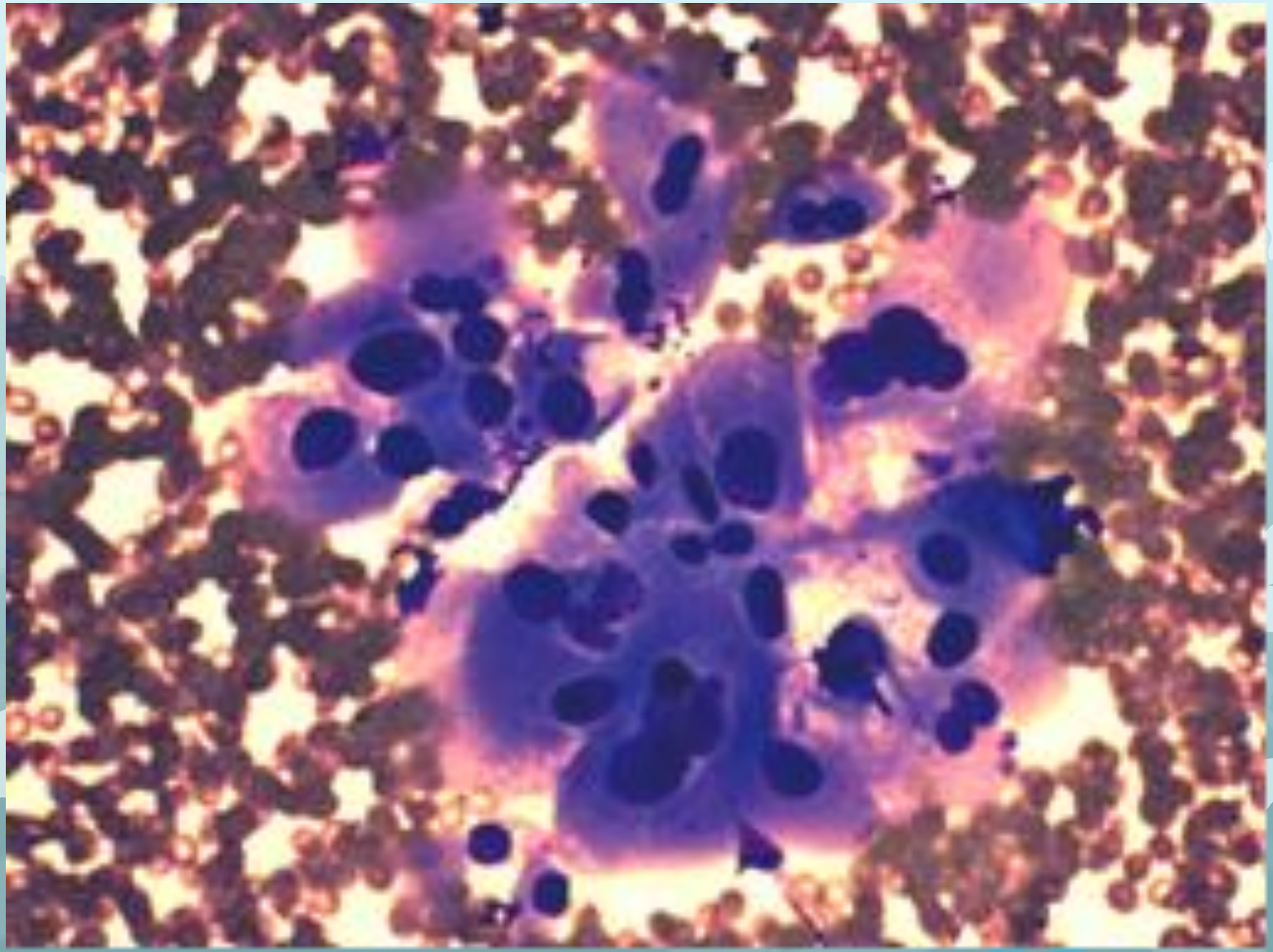
Final Cytological Diagnosis for Case 5

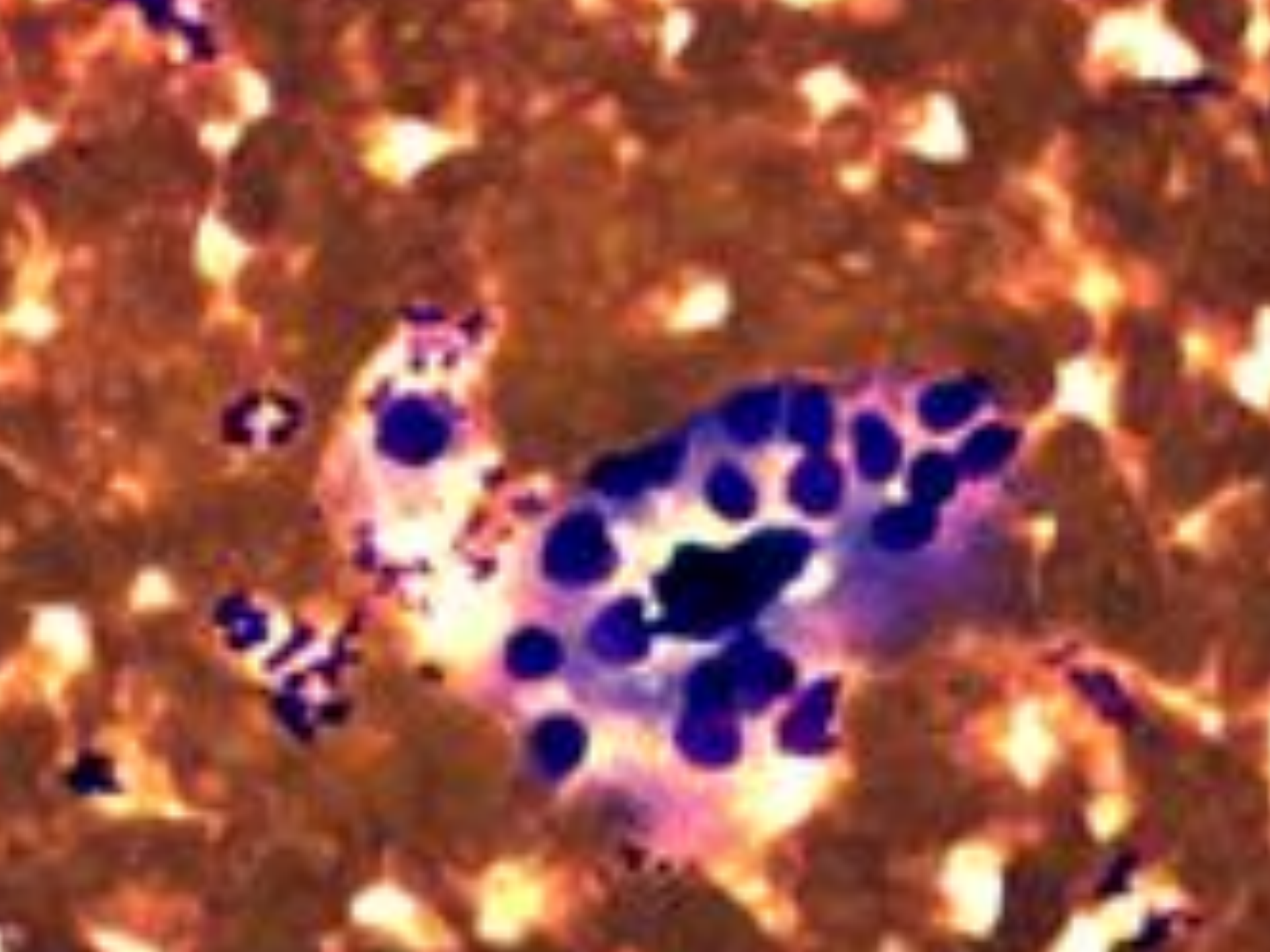
Suspicious for a follicular neoplasm
(Bethesda category IV)

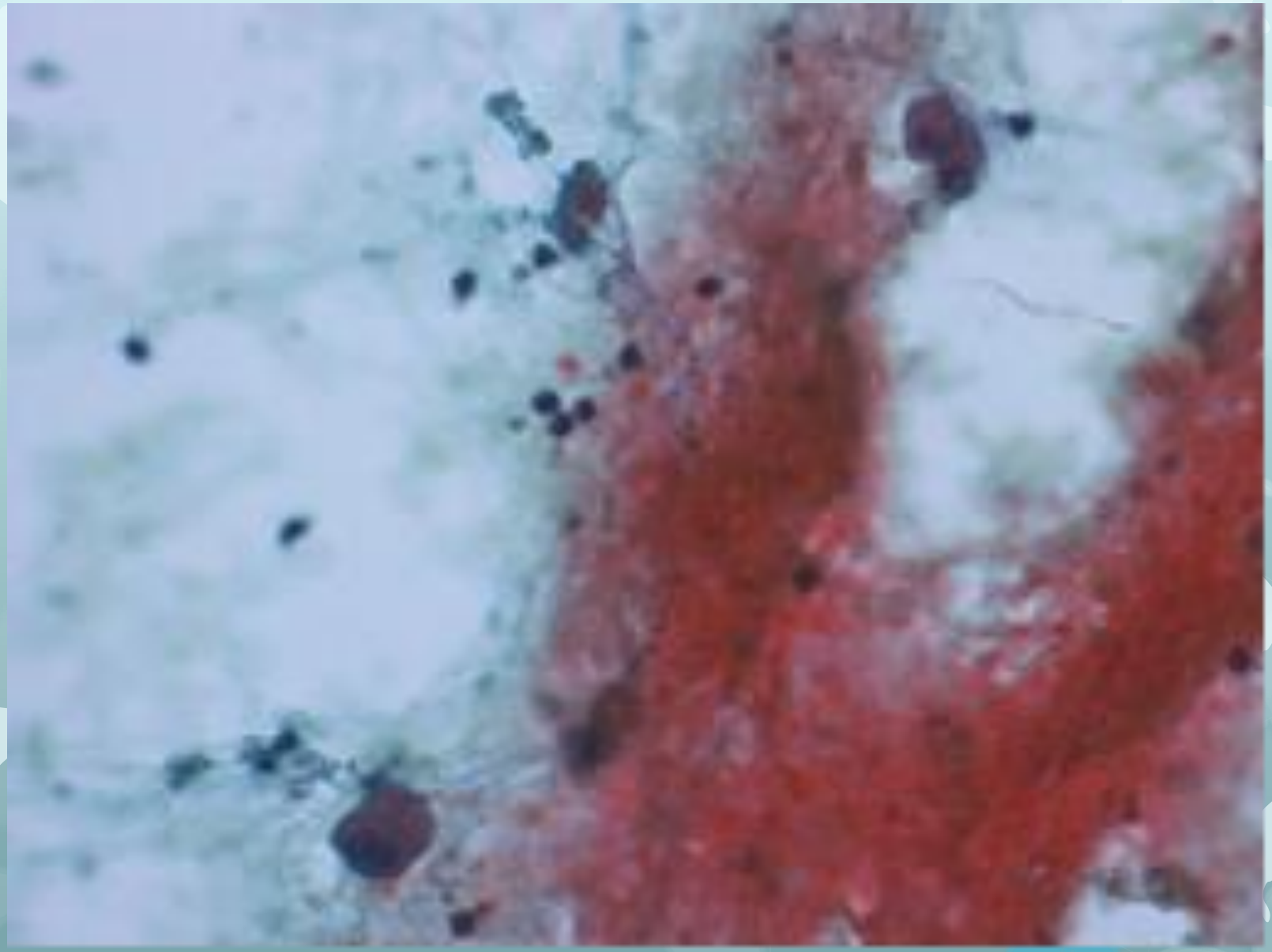
Case 6
Thyroid right upper pole nodule with increased blood flow

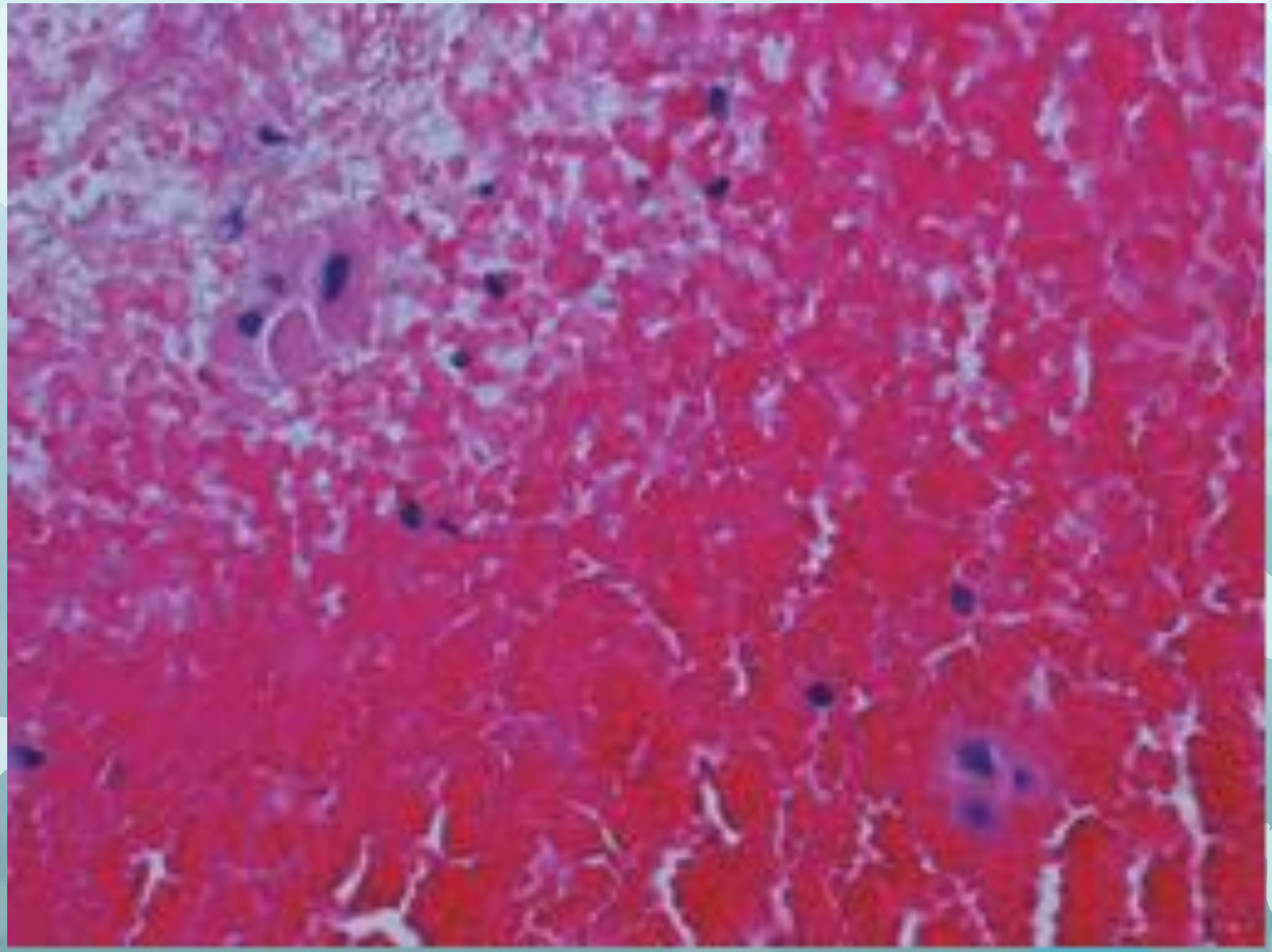










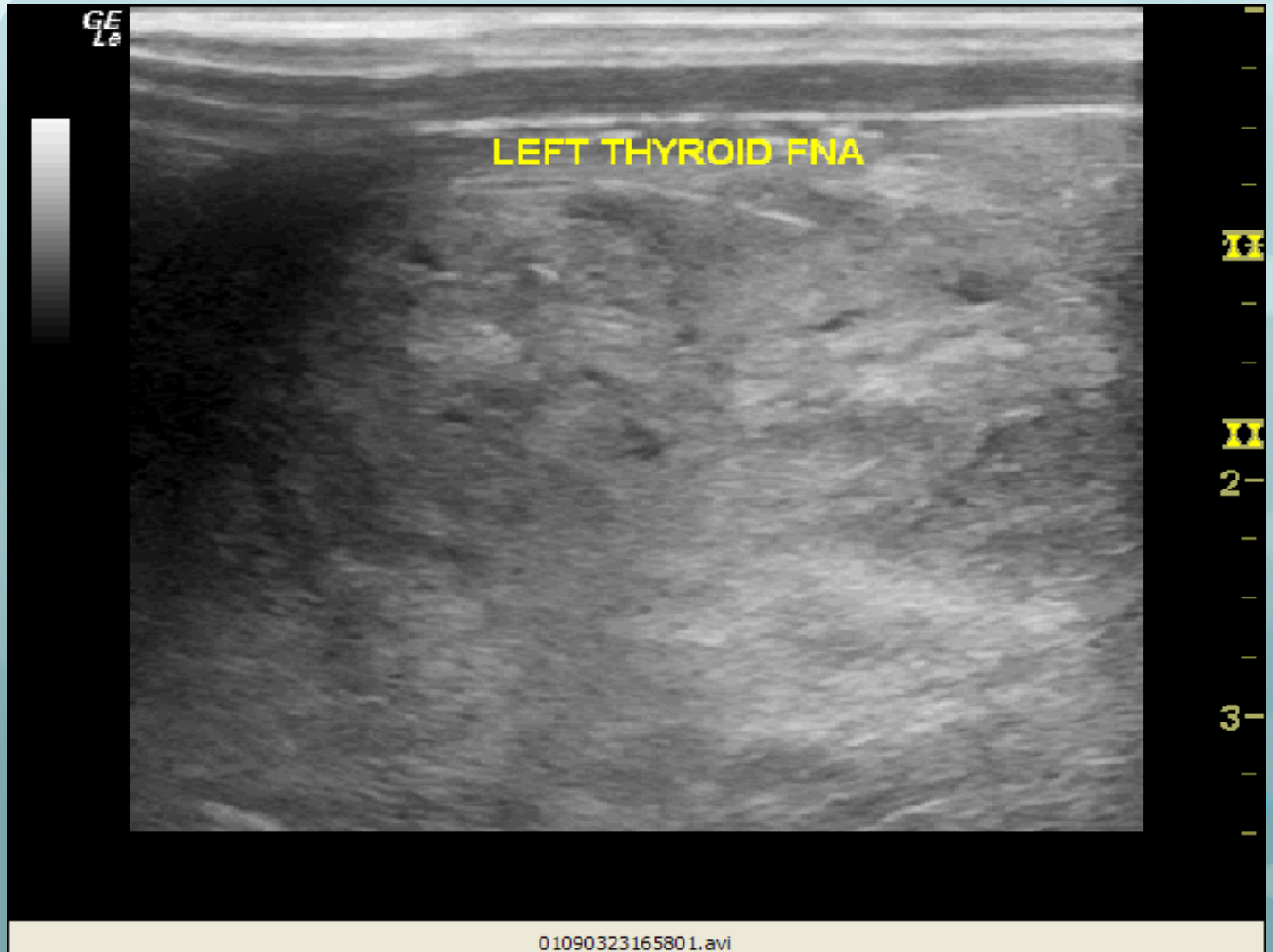


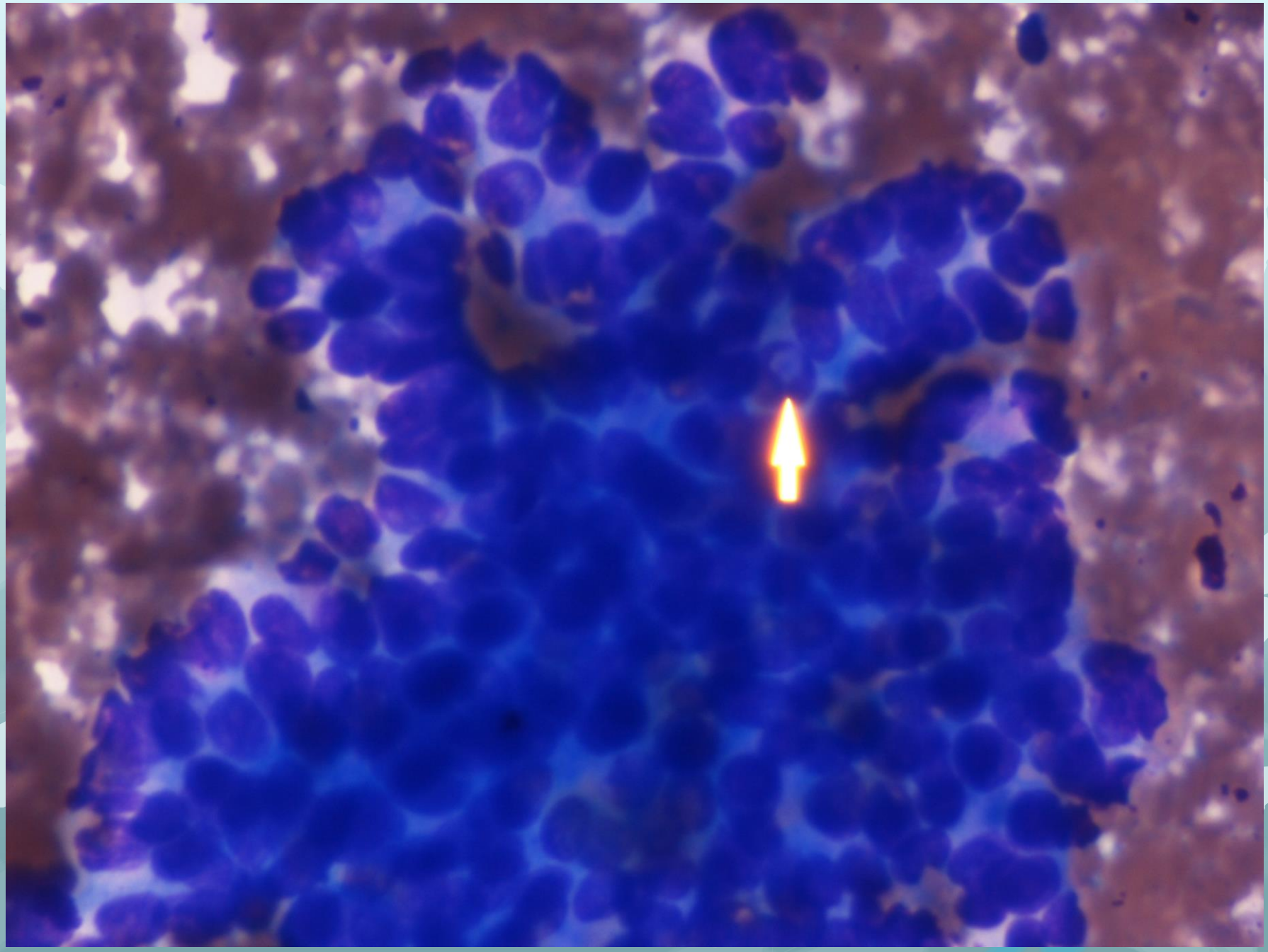
Final Cytological Diagnosis for Case 6

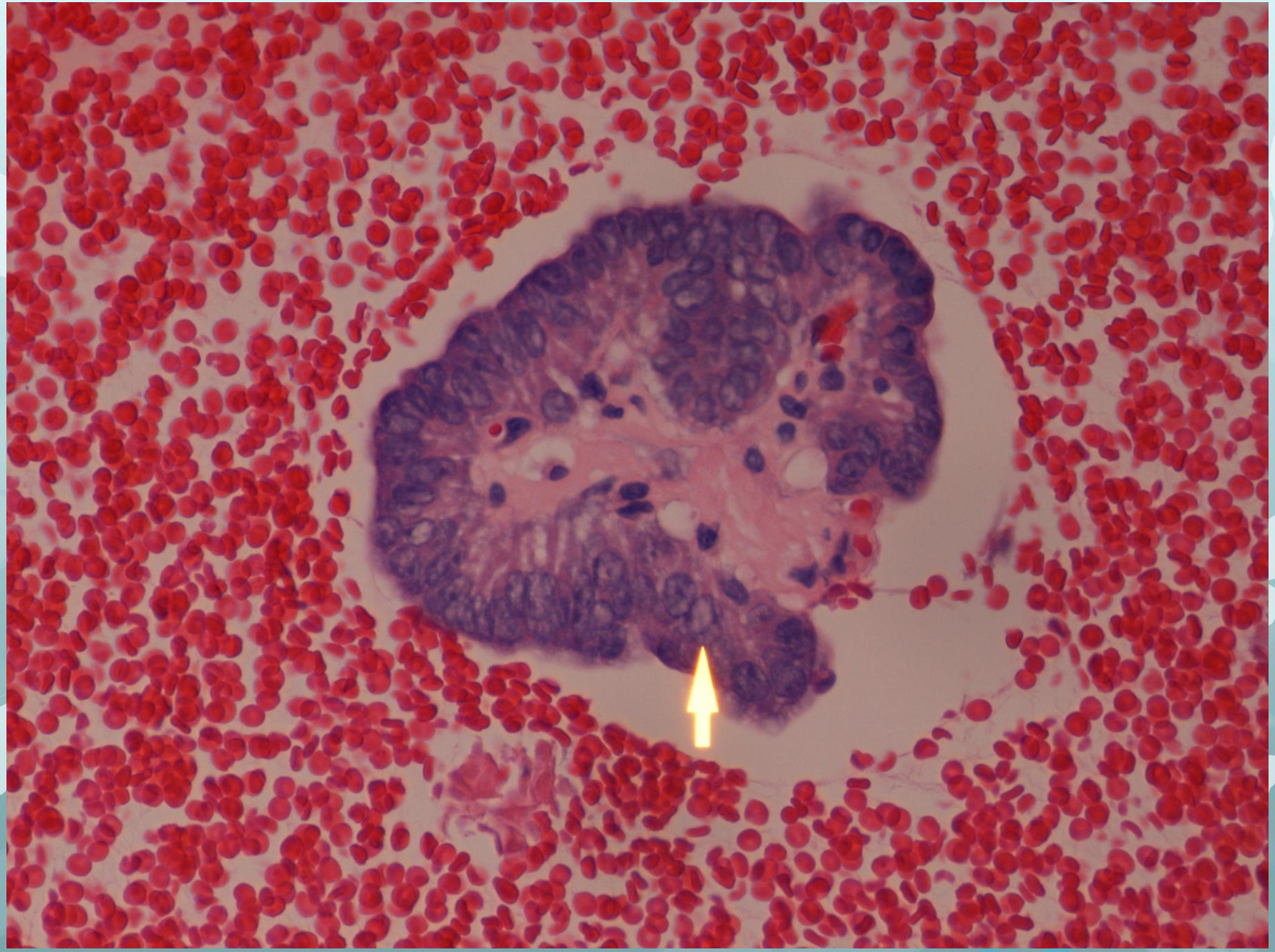
Oncocytic/Hurthle cell lesion, suspicious for neoplasm (Bethesda Category IV)

Case 7

65 male with 6.7cm LT thyroid mass (PC09-9813)





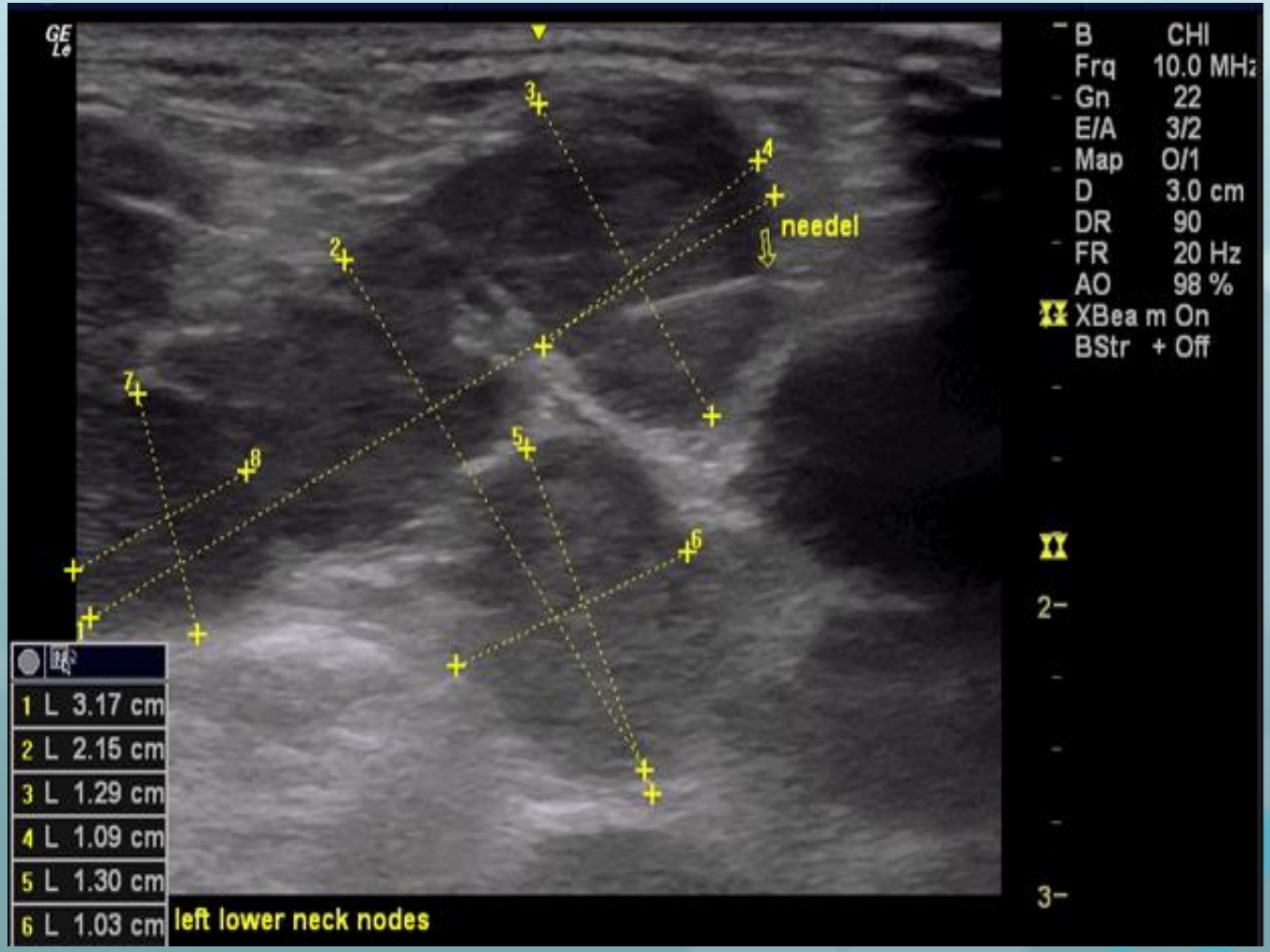


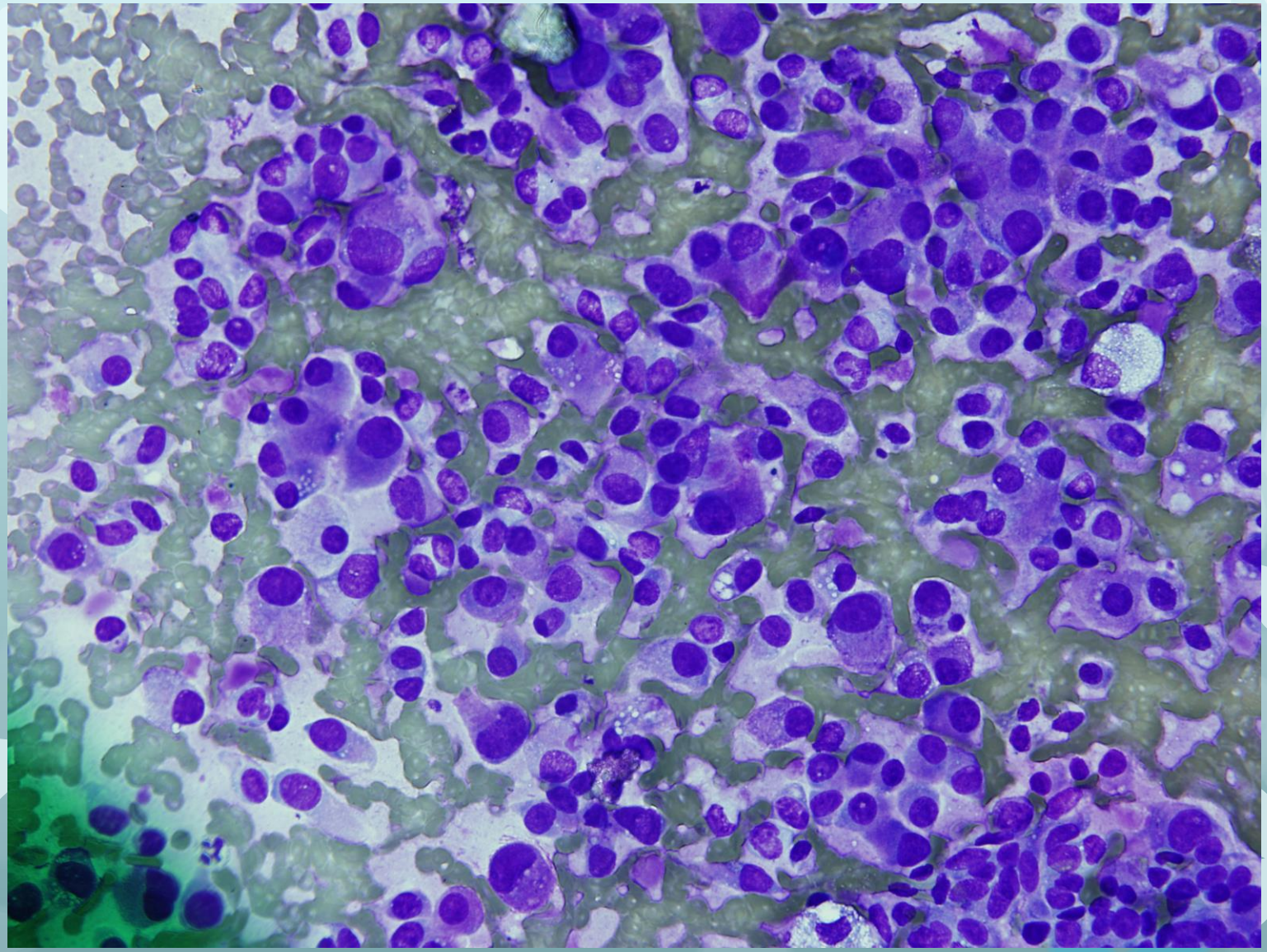
Final Cytological Diagnosis for Case 7

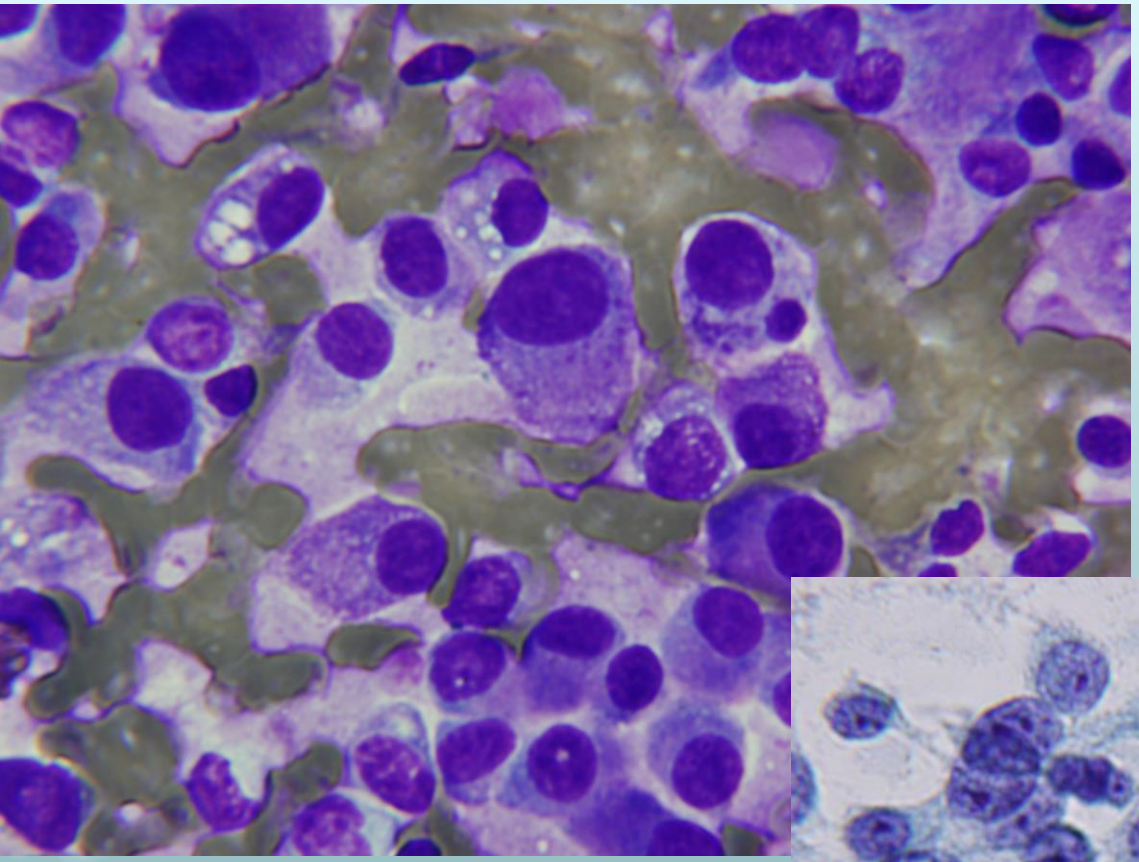
PTC

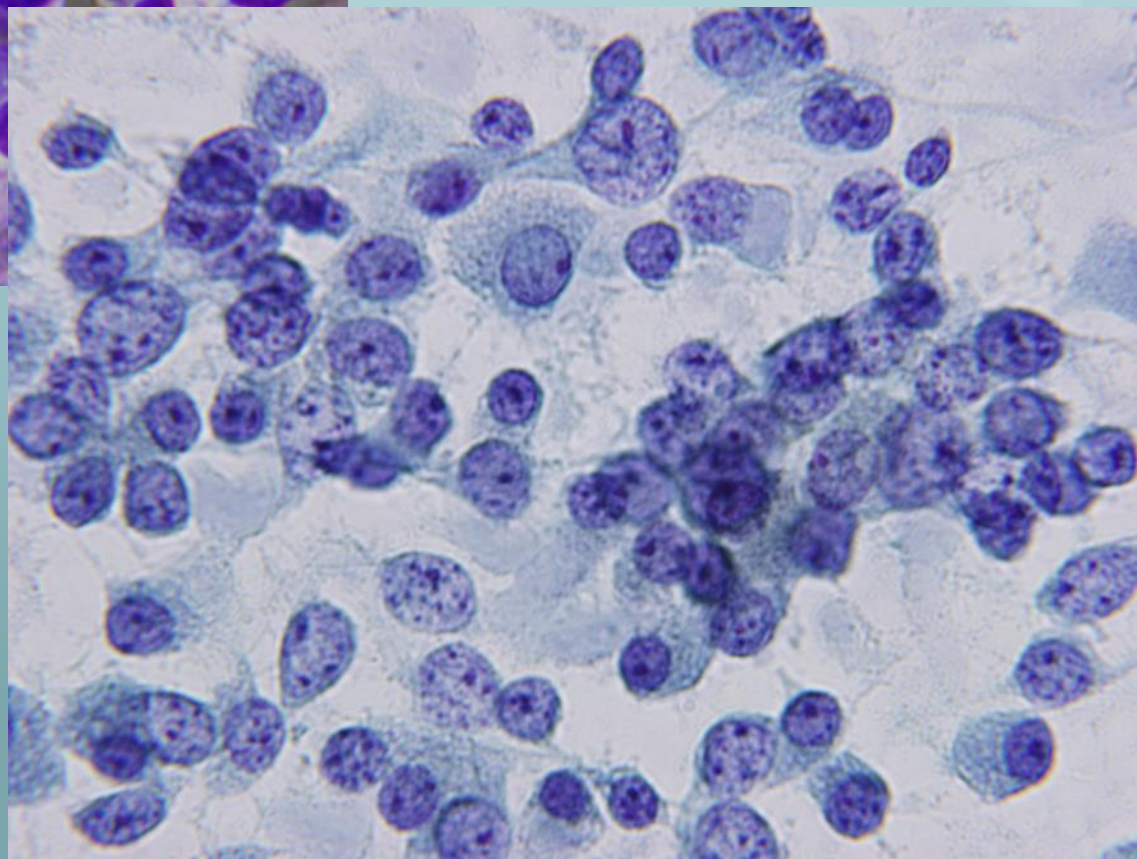
(Bethesda Category VI)

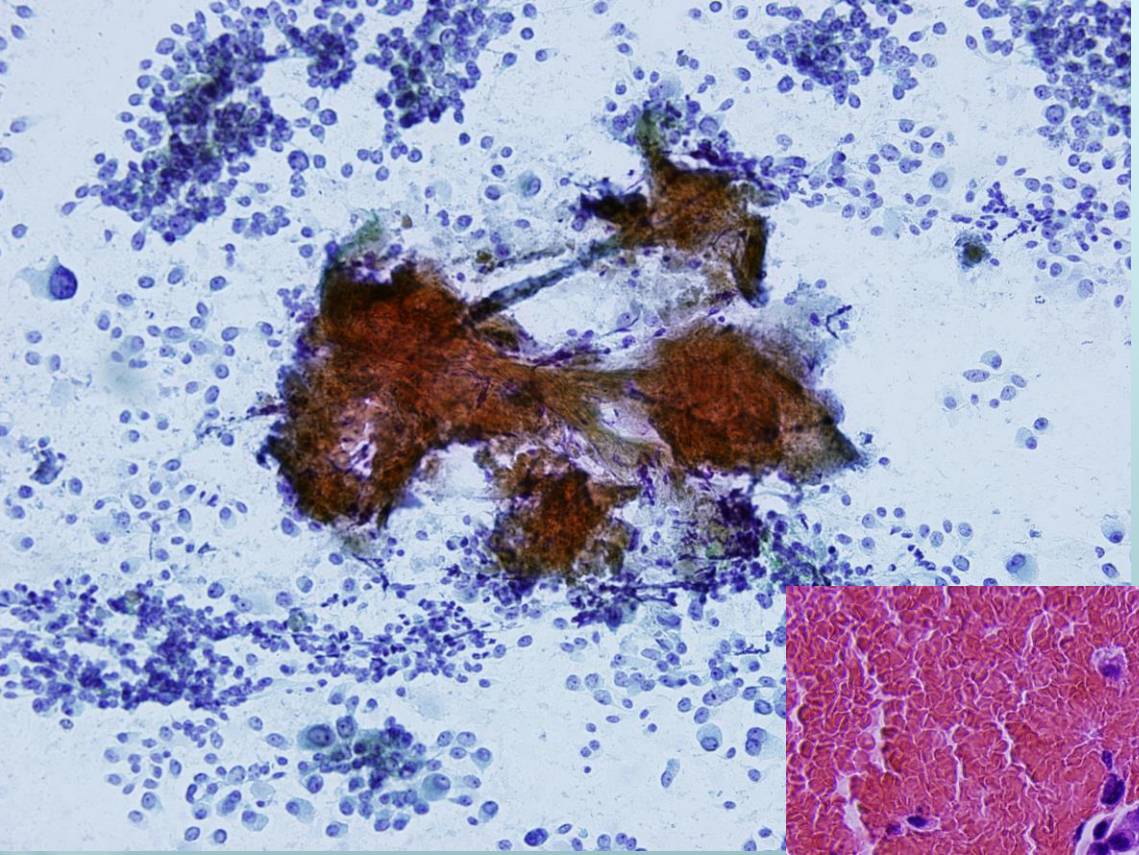
Case 8
35 year-old male with thyroid and neck nodular masses

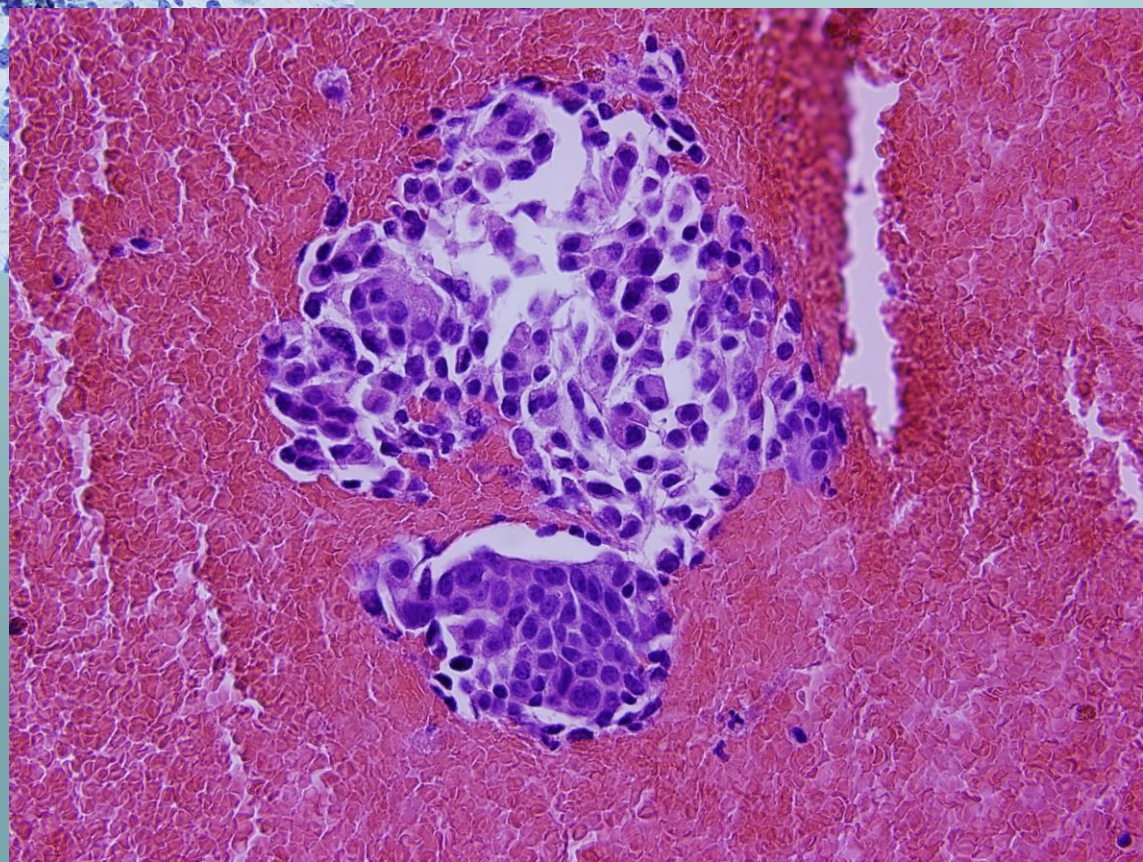




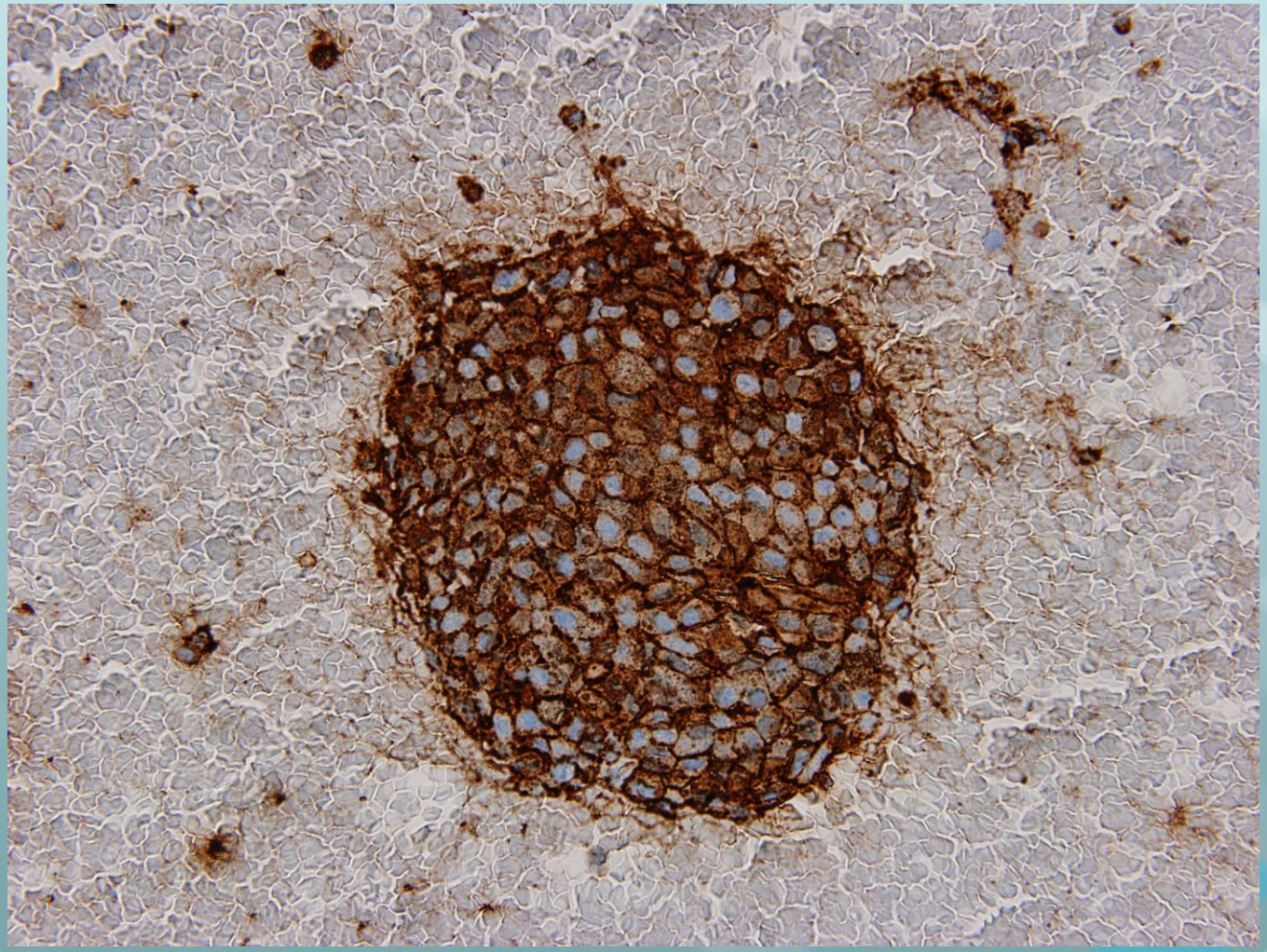




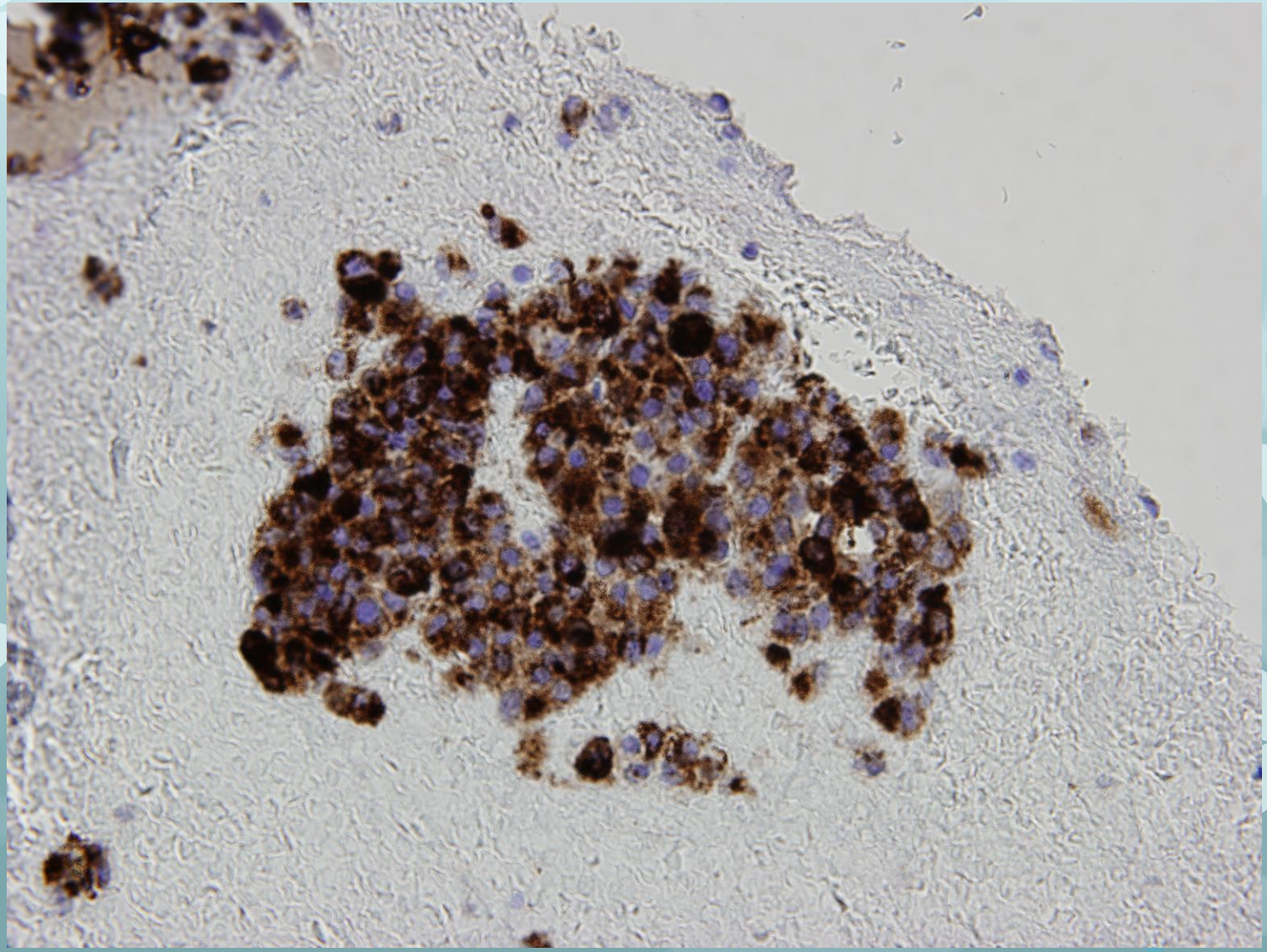




Chromogranin



CEA



Final Cytological Diagnosis for Case 8

Metastatic Medullary Thyroid Carcinoma

(Bethesda Category VI)

Ultrasound guided FNA of thyroid performed by cytopathologists enhances Bethesda diagnostic value

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The authors declare no conflict of interest

Abstract

Background: Ultrasound (US) guided fine needle aspiration (FNA) biopsy of thyroid can be performed by either a radiologist, an endocrinologist or a cytopathologist. All samples are examined and reported by cytopathologists based on The Bethesda System for Reporting Thyroid Cytopathology (BS). One question is whether there is any performer-dependent difference. This study is designed to answer such a question.

Design: 651 thyroid US-FNA cases including 283 performed by cytopathologists and 368 by non-cytopathologists during the period of 09/01/2010 to 5/30/2012. All the cases from the non-cytopathologist group were done without immediate cytological evaluation. The cases from the cytopathologist group were all performed, with onsite evaluation and finally signed out by cytopathologists. For each case, BS was applied for diagnostic classification. The cases were also correlated with surgical follow-up (SFU). The statistical analysis for the cases with SFU was made by using the surgical pathology diagnosis as the gold standard.

Results: Among the 283 cases performed by cytopathologists, there were 8(2.8%) non-diagnostic/unsatisfactory (BS 1), 197(69.6%) benign (BS 2), 31(11%) atypical/follicular lesion of undetermined sigaificance (BS 3), 14(5%) follicular neoplasm (FN)/suspicious for FN (BS 4), 12(4.2%) suspicious for maligaancy (BS 5), and 21(7.4%) malignant (BS 6), and there were 55(19.4%) cases with SFU. The 368 cases performed by others showed 76 (21%) BS 1, 238 (65%) BS 2, 26 (7%) BS 3, 10 (3%) BS4, 9 (2.5%) BS 5, and 9 (2.5%) BS6, and there were 26 (7%) cases with SFU. In comparison, the cytopathologist-performed group showed fewer unsatisfactory cases (2.8% vs. 21%); considerably higher percentage of cases falling in to BS2-6; and markedly high rate of SFU (19.4% vs.7%). The Statistical results based on SFU revealed that the cytopathologist group achieved better sensitivity (91.3% vs. 78%); better PPV (87.5% vs. 70%); similar NPV (88.2% vs. 88%); slightly better specificity (83.3% vs. 82%); and better overall accuracy (87.8% vs. 81%) compared with the non-cytopathologist group.

Conclusion: US-FNA performed by cytopathologists showed a lower unsatisfactory rate, higher rate of SFU, higher sensitivity, better PPV and greater overall accuracy. Having actual hands-on experience gives cytopathologists more precise knowledge of the lesion before aspiration. Onsite cytological evaluation can help to triage a specimens appropriately. Whereas in the non-pathologist group, some information may have been lost between the aspiration and interpretation.

Background: Ultrasound (US) guided fine needle aspiration (FNA) biopsy of thyroid can be performed by either a radiologist, an endocrinologist or a cytopathologist (Fig. 1). All samples are examined and reported by cytopathologists based on The Bethesda System for Reporting Thyroid Cytopathology (BS). One question is whether there is any performer-dependent difference. This study is designed to answer such a question.

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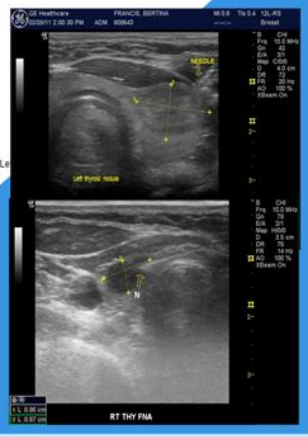


Figure 1 – Ultrasound Guided FNA Biopsy of Thyroid

US-FNA performed by	Total # of cases	# of SFU	BS 1	BS 2	BS 3	BS 4	BS 5	BS 6
Cytopathologists	283	55 (19.4%)	8 (2.8%)	197 (69.6%)	31 (11%)	14 (5%)	12 (4.2%)	21 (7.4%)
Non-cytopathologists	368	26 (7%)	76 (21%)	238 (65%)	26 (7%)	26 (7%)	9 (2.5%)	9 (2.5%)

Table 1: Comparative **BS** diagnostic rate between cytopathologist-performed and non-cytopathologist-performed Thyroid FNA

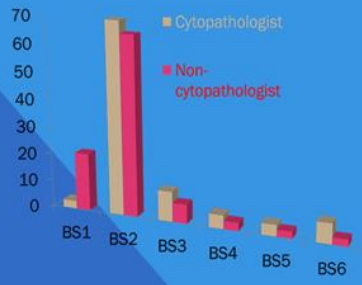


Figure 2. Comparative BS Diagnostic rate (%)

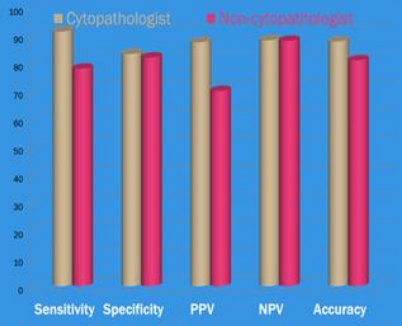
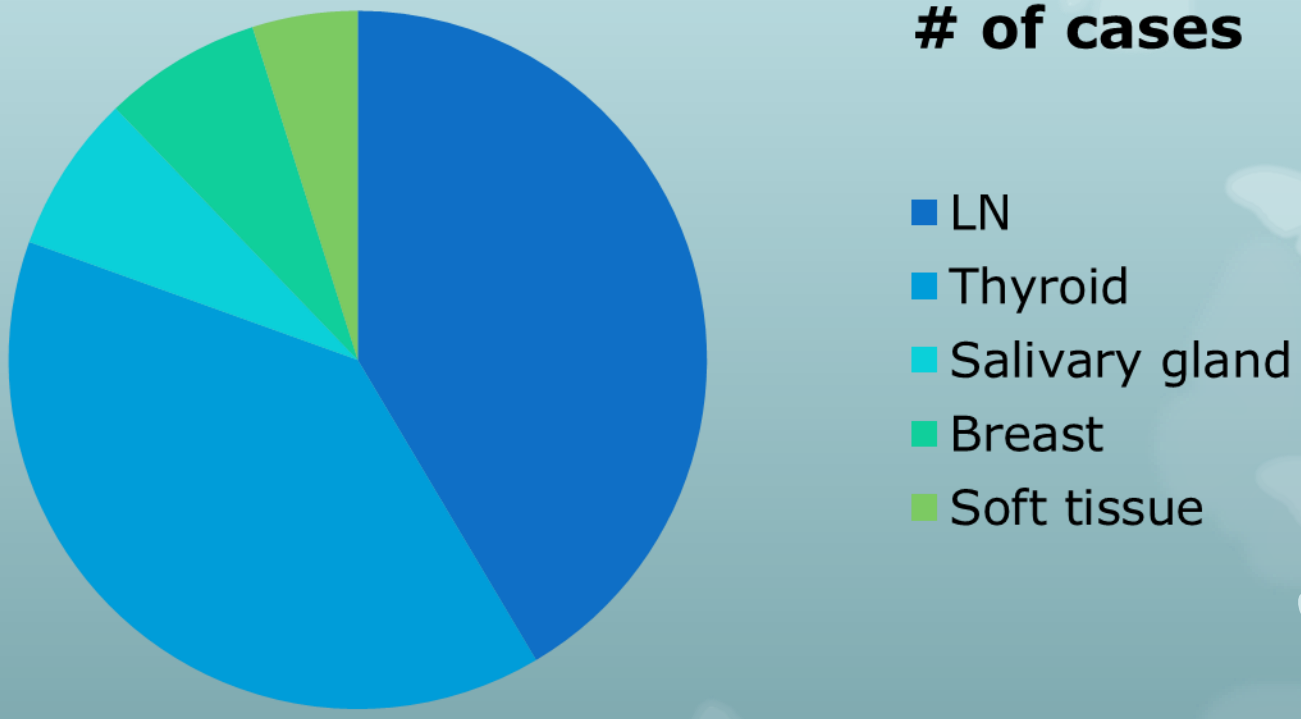


Figure 3. The Statistical results based on SFU

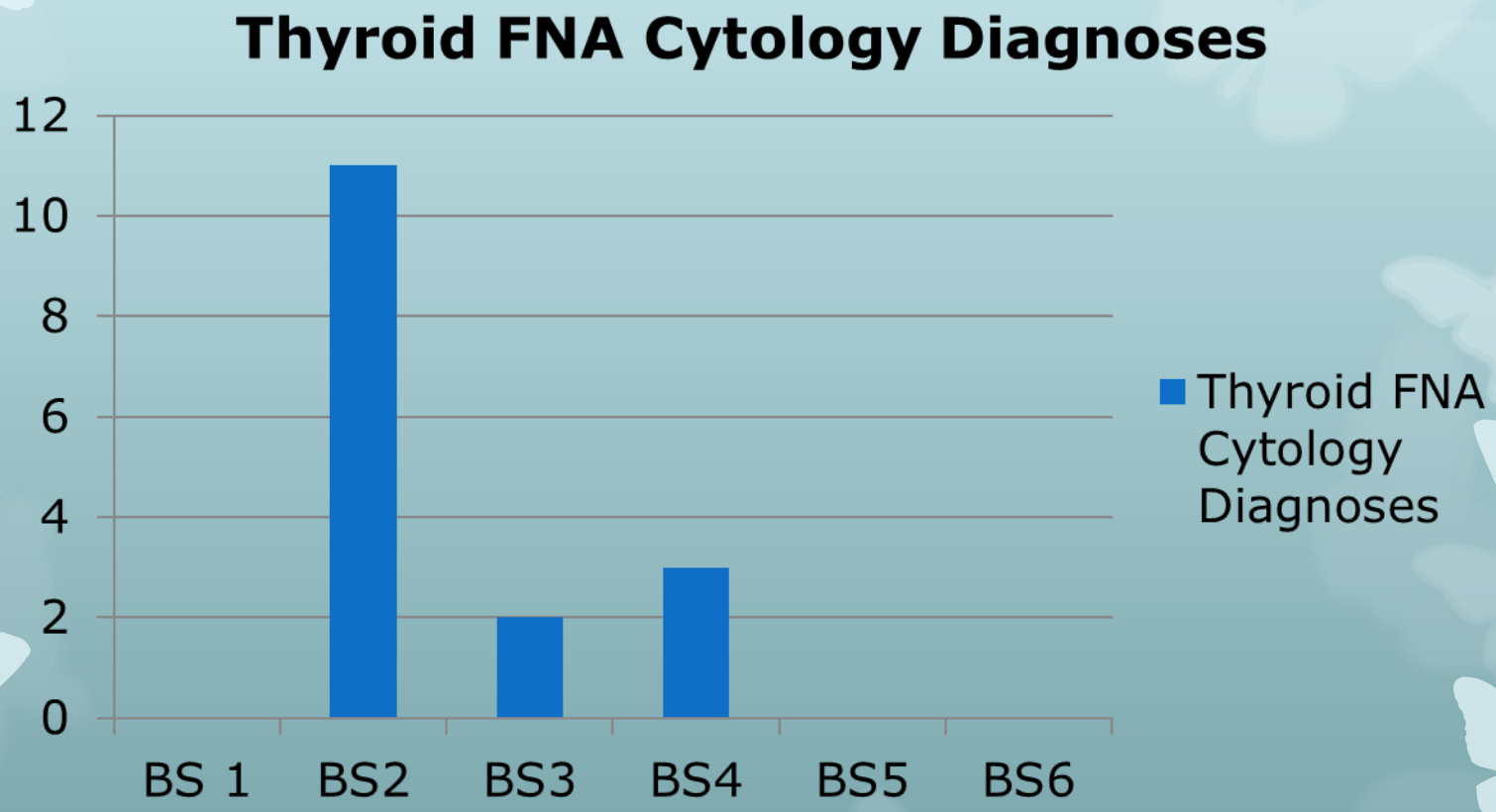
Results: Among the 283 cases performed by cytopathologists, there were 8(2.8%) non-diagnostic /unsatisfactory (BS 1), 197(69.6%) benign (BS 2), 31(11%) atypical/follicular lesion of undetermined significance (BS 3), 14(5%) follicular neoplasm (FN)/suspicious for FN (BS 4), 12(4.2%) suspicious for malignancy (BS 5), and 21(7.4%) malignant (BS 6), and there were 55(19.4%) cases with SFU. The 368 cases performed by others showed 76 (21%) BS 1, 238 (65%) BS 2, 26 (7%) BS 3, 10 (3%) BS4, 9 (2.5%) BS 5, and 9 (2.5%) BS6, and there were 26 (7%) cases with SFU (Table 1). In comparison, the cytopathologist-performed group showed fewer unsatisfactory cases (2.8% vs. 21%); considerably higher percentage of cases felling in to BS2-6; and markedly high rate of SFU (19.4% vs.7%) (Figure 2). The Statistical results based on SFU revealed that the cytopathologist group achieved better sensitivity (91.3% vs. 78%); better positive predictive value (PPV) (87.5% vs. 70%); similar negative predictive value (NPV) (88.2% vs. 88%); slightly better specificity (83.3% vs. 82%); and better overall accuracy (87.8% vs. 81%) compared with the non-cytopathologist group (Figure 3).

Conclusion: US-FNA performed by cytopathologists showed a lower unsatisfactory rate, higher rate of SFU, higher sensitivity, better PPV and greater overall accuracy. Having actual hands-on experience gives cytopathologists more precise knowledge of the lesion before aspiration. Onsite cytological evaluation can help to triage a specimen appropriately. Whereas in the non-pathologist group, some information may have been lost between the aspiration and interpretation.

US-FNA at Stony Brook (N=41)



US-FNA of Thyroid at Stony Brook (N=16)



How are we going to manage cytologically Indeterminate Thyroid Nodules at Stony Brook?

Bethesda Thyroid System	Risk of cancer (%)	Usual management
I: Non diagnostic	1-4%	Repeat FNA -US
II: Benign	0-3%	Clinical f/u
III: Follicular lesion or atypia of undetermined significance	5-15%	Repeat FNA
IV: Follicular Neoplasm or suspicious for follicular neoplasm	15-30%	Lobectomy
V: Suspicious for malignancy	60-75%	Thyroidectomy or lobectomy
VI: Malignant	97-99%	Thyroidectomy

Management of Indeterminate FNAs – Molecular Approaches

Veracyte Afirma Gene Classifier

(rule out)

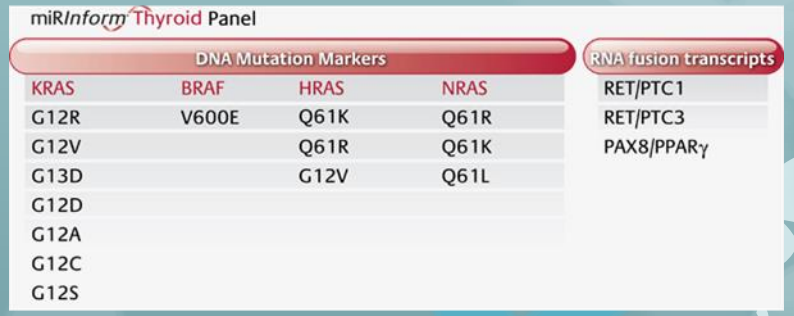
The miRInform Molecular Panel (Interpace Diagnostics /Asuragen) (rule in)

ThyroSeq (University of Pittsburgh)

Afirma VS. miRInform

- Afirma is to rule out cancer (**Sensitivity**)
- Identifies benign nodules by measuring expression of 167 genes via messenger RNA (mRNA),

- miRInform identifies malignancy (**Specificity**)
- 17 known genetic alterations in thyroid cancer and adds analysis of a panel of micro RNAs (miRNA) to label a nodule as cancerous



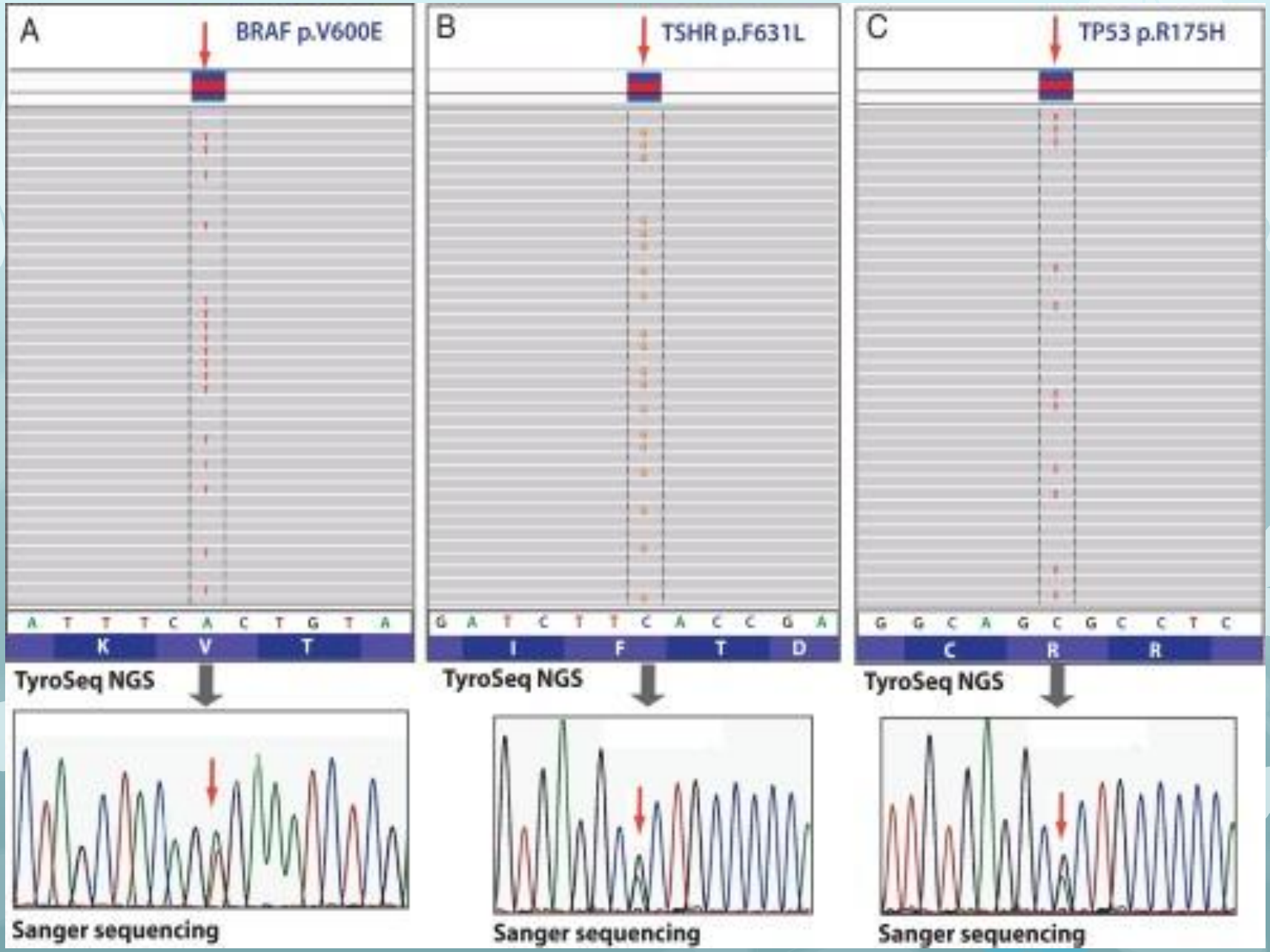
ThyroSeq Panel

- Next Generation Sequencing (NGS)
- Customized an IonTorrent platform to look for Thyroid Cancer related point mutation (14) and gene fusions (42) in >1000 hotspots
- Gene List for Mutations: *AKT1, BRAF, CTNNB1, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TP53, TSHR, TERT, EIF1AX*
- Gene List for Gene Fusions and Gene Expression: *RET, PPARG, NTRK1, NTRK3, ALK, IGF2BP3, BRAF, MET, CALCA, PTH, SLC5A5, TG, TTF1, KRT7, KRT20*

Table 1.

Genes and Exons Included in ThyroSeq Panel

Chromosomes	Genes
chr1	NRAS exons 2, 3
chr3	CTNNB1 exon 3
chr3	PIK3CA exons 9, 20
chr7	BRAF exon 15
chr10	RET exons 10, 11, 12, 13, 15, 16
chr10	PTEN exons 5, 6, 7, 8
chr11	HRAS exons 2, 3
chr12	KRAS exons 2, 3
chr14	TSHR exon 10
chr14	AKT1 exon 3


A
BRAF p.V600E
A T T T C A C T G T A
K V T
TyroSeq NGS
Sanger sequencing
B
TSHR p.F631L
G A T C T T C A C C G A
I F T D
TyroSeq NGS
Sanger sequencing
C
TP53 p.R175H
G G C A G C G C C T C
C R R
TyroSeq NGS
Sanger sequencing

Study (N=228) by UPMC

- ThyroSeq DNA assay identified mutations in:
  - 19 of 27 of PTC (70%)
  - 25 of 30 follicular variant PTCs (83%)
  - 3 of 10 poorly differentiated carcinomas (30%)
  - 20 of 27 anaplastic (ATCs) (74%)
  - 11 of 15 medullary thyroid carcinomas (73%)
- 5 of 83 benign nodules (6%) were positive for mutations

Nikiforov YE et al, Clin Thyroidol 2013;25:288–289 Next-Generation Sequencing Has Identified New Oncogenic Mutations in Thyroid Nodules

A prospective analysis of indeterminate FNA samples (N=1056)

Risk of malignancy based on cytology:

- AUS/FLUS = 14%
- FN/SFN = 27%
- SMC =54%

Risk of malignancy if + for any mutations in panel:

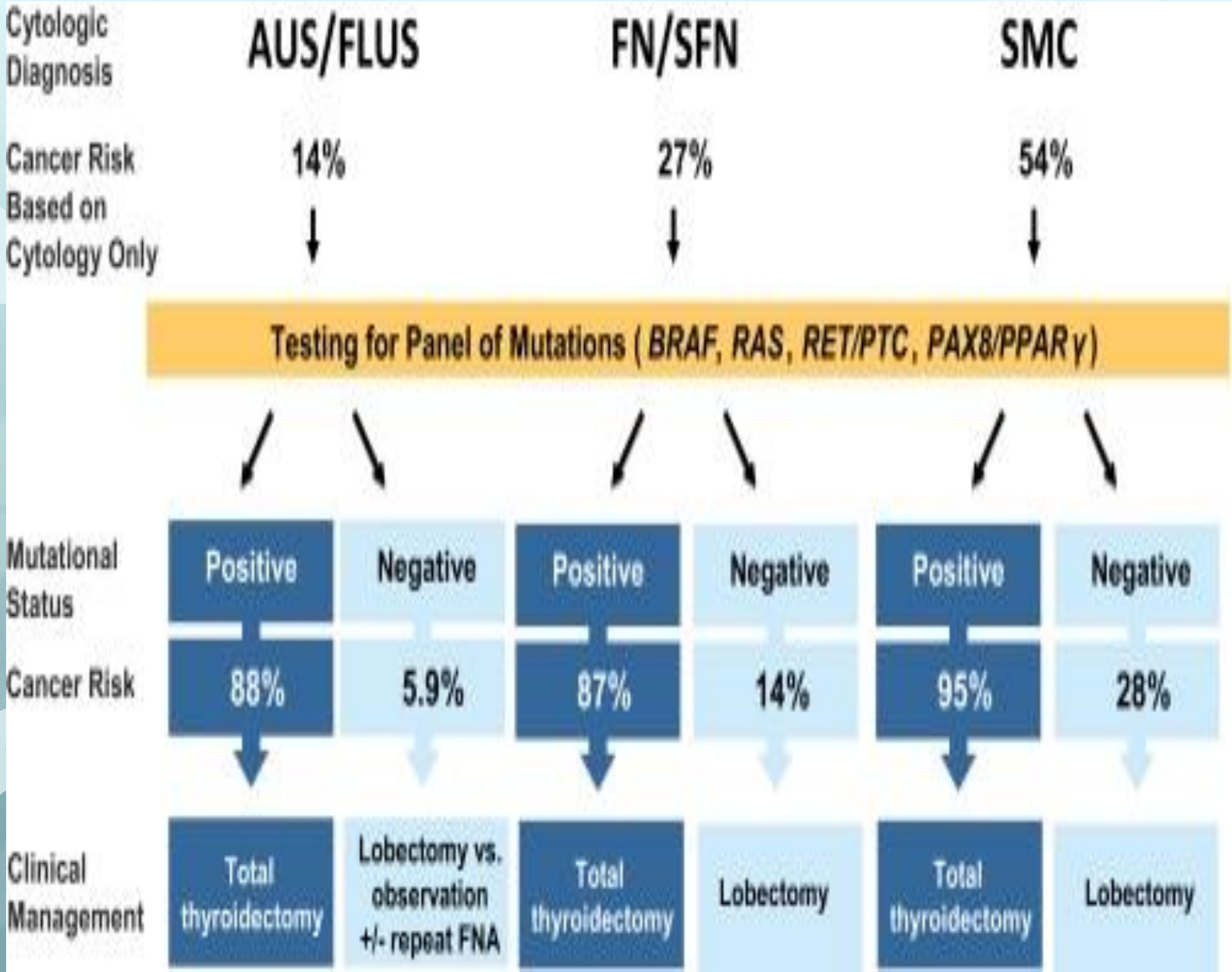
- AUS/FLUS = 88%
- FN/SFN = 87%
- SMC = 95%

Nikiforov YE et al. 2011, J Clin Endocrinol Metab 96:3390–3397

Atypia of undetermined significance/Follicular lesions of undetermined significance (AUS/FLUS) (n=247)			
	Histology Malignant (n=35)	**Histology Benign (n=212)**	
Mutation Positive (n=25)	16 *RAS* (16 PTC,FV) 5 *BRAF* (4 PTC, 1 PTC,FV) 1 *PAX8/PPARg* (1 PTC,FV)	3 *RAS* (3 FA)	***Sensitivity*** **63%** ***Specificity*** **99%** ***PPV*** **88%** ***NPV*** **94%** ***Accuracy*** **94%**
Mutation Negative (n=222)	13 (11 PTC, FV, 2 PTC)	209 (166 HN, 43 FA)	

Follicular or Hürthle cell neoplasm/Suspicious for follicular neoplasm (FN/SFN) (n=214)			
	Histology Malignant (n=58)	**Histology Benign (n=156)**	
Mutation Positive (n=38)	2 *BRAF* (1 PTC, 1 PTC,FV) 29 *RAS* (21 PTC,FV, 5 PTC, 3 FTC) 2 *PAX8/PPARg* (2 PTC,FV)	5 *RAS* (5 FA)	***Sensitivity*** **57%** ***Specificity*** **97%** ***PPV*** **87%** ***NPV*** **86%** ***Accuracy*** **86%**
Mutation Negative (n=176)	25 (16 PTC,FV, 3 PTC, 6 FTC)	151 (95 HN, 56 FA)	

Suspicious for malignant cells (SMC) (n=52)			
	Histology Malignant (n=28)	**Histology Benign (n=24)**	
Mutation Positive (n=20)	10 *BRAF* (10 PTC) 7 *RAS* (6 PTC,FV, 1 FTC) 1 *PAX8/PPARg* (1 FTC) 1 *RET/PTC* (1 PTC)	1 *RAS* (1 FA)	***Sensitivity*** **68%** ***Specificity*** **96%** ***PPV*** **95%** ***NPV*** **72%** ***Accuracy*** **81%**
Mutation Negative (n=32)	9 (7 PTC, 2 PTC,FV)	23 (17 HN, 6 FA)	


Cytologic Diagnosis
AUS/FLUS
FN/SFN
SMC
Cancer Risk Based on Cytology Only
14%
27%
54%
Testing for Panel of Mutations (BRAF, RAS, RET/PTC, PAX8/PPARγ)
Mutational Status
Positive
Negative
Positive
Negative
Positive
Negative
Cancer Risk
88%
5.9%
87%
14%
95%
28%
Clinical Management
Total thyroidectomy
Lobectomy vs. observation +/- repeat FNA
Total thyroidectomy
Lobectomy
Total thyroidectomy
Lobectomy

Another Study performed with NGS (N=34 FNAs)

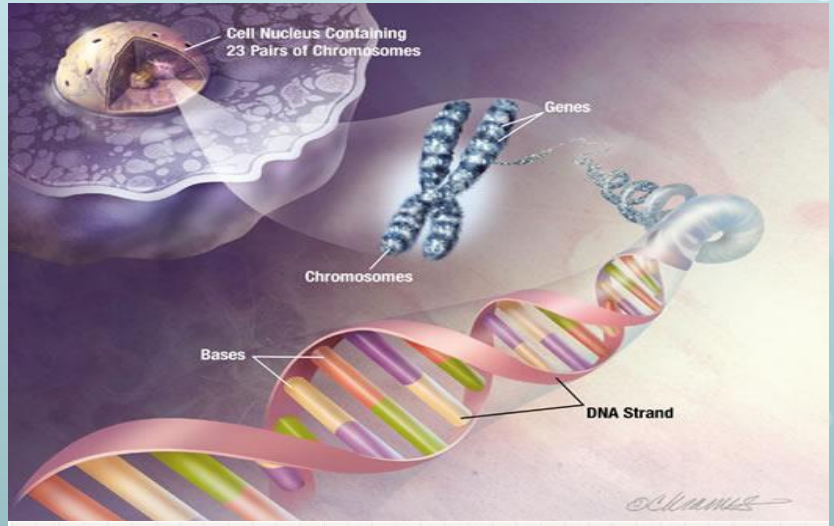
- Marie Le Mercier, PhD, a pathologist at the ULB-Erasme Hospital in Brussels used the IonTorrent AmpliSeq Cancer Hotspot Panel to classify indeterminate nodules.
- 50 genes known to be associated with thyroid cancers, including BRAF and the RAS family of mutations.
- Retrospective study
  - 71% Sensitivity for malignant nodules
  - 93% Specificity for benign nodules with no mutations

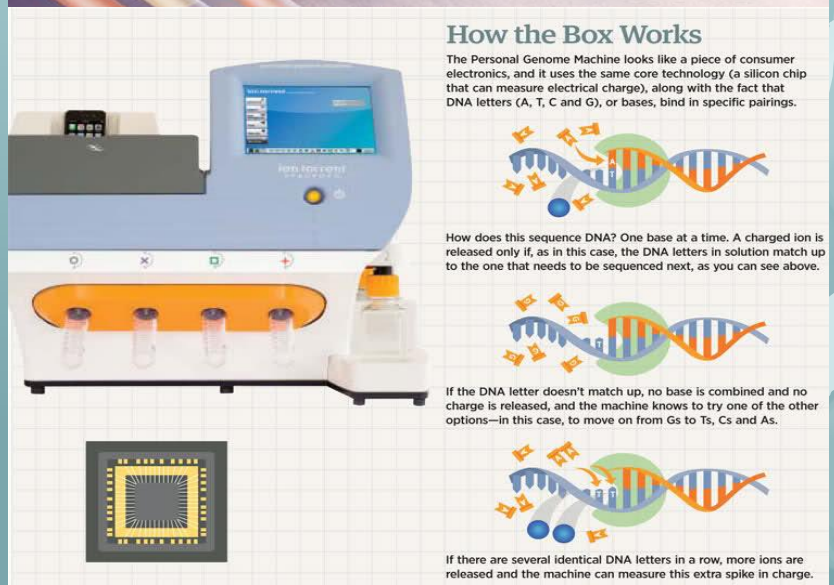
Marie Le Mercier1, Nicky D'Haene1, Nancy De Nève1, Oriane Blanchard1, Caroline Degand1, Sandrine Rorive1,2 andIsabelle Salmon1,2,* "Next-generation sequencing improves the diagnosis of thyroid FNA specimens with indeterminate cytology" Histopathology Volume 66, Issue 2, pages 215–224, January 2015
Article first published online: 10 NOV 2014 | DOI: 10.1111/his.12461

Conclusions

- Cytopathologists are able to perform ultrasound-guided Thyroid FNA and to enhance Bethesda diagnostic values
- New molecular methods for indeterminate FNA samples seem to be promising
- Stony Brook Cytopathologists provide comprehensive Thyroid FNA Cytology-Molecular Service

Stony Brook Cytopathology US-FNA Service





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