

TIROIDE (1)

Citologia tiroidea e biologia molecolare: fra classificazioni e azioni cliniche

Moderatori

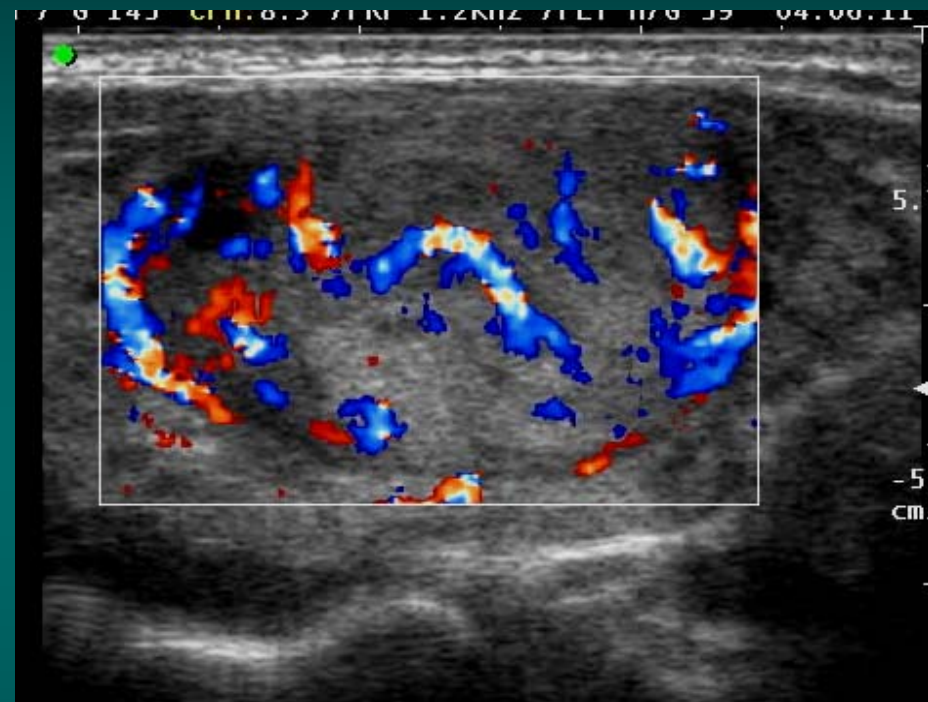
H. Gharib, E. Papini

- **Classificazioni citologiche: verso uno schema internazionale unificato?**
A. Crescenzi
- **Problemi aperti**
 - **Microistologia: come quando e perché?**
P. Trimboli
 - **Biologia molecolare su agoaspirato tiroideo: ready for prime time?**
G. Fadda
- **Gene expression classifiers: cost and efficacy**
H. Gharib
- **Dal caso clinico all'azione, discussione guidata: Speaker**
A. Frasoldati
Discussants
F. Nardi, M. Torlontano
- **Take-home messages**
H. Gharib, E. Papini

Clinical Case 1.



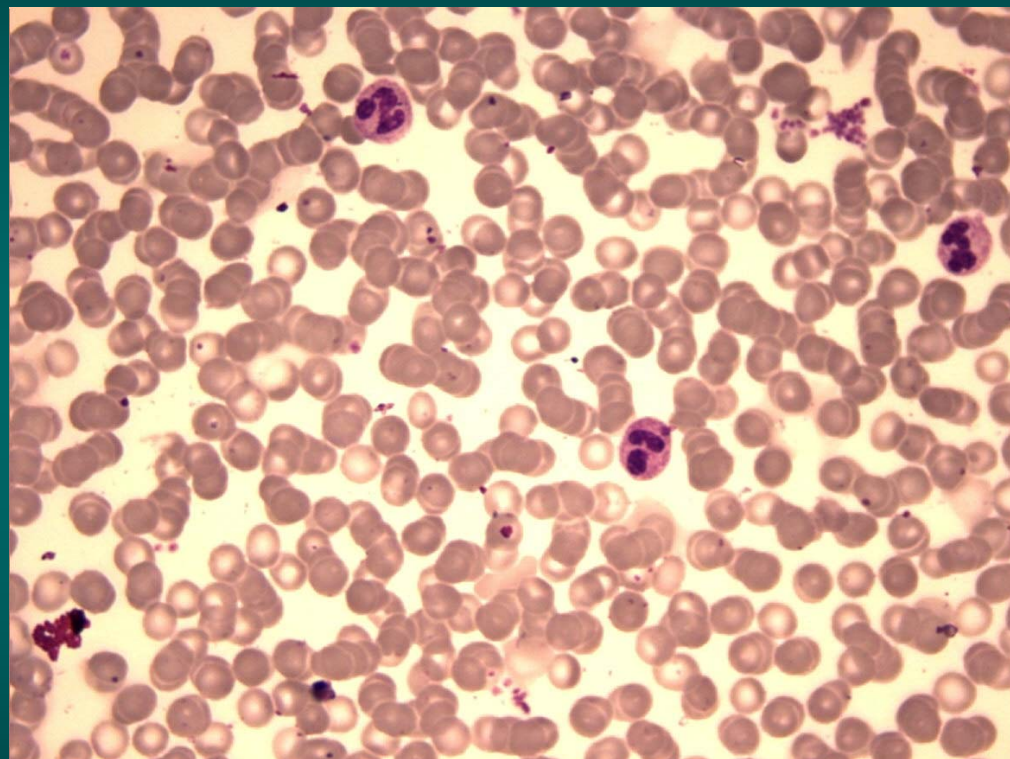
- *Marta, age 31*
- *Chronic autoimmune thyroiditis (thyroid screening during pregnancy)*
- *TSH 3.5 uU/ml, TPOAb 366 U/ml.*
- *L-thyroxine treatment: 50 ug/day*
- *Six month after delivery: thyroid US examination.*



16 mm Ø hypo-hypoechoic nodule, inhomogeneous texture, slightly irregular margins, peripheral and internal vascularization.

Clinical Case 1. FNA report (1)

Non diagnostic
(blood contamination, scant
thyrocyte aggregates,
scarce colloid).



SIAPEC Reporting system: "TIR 1"

Case 1. Question time: Round 1.



- Which are the reasons for a “non diagnostic” thyroid FNA ?
- Possible solutions?

The TIR 1 challenge: possibile causes

Tir-1 occurrence depends upon the characteristics of the lesion, technical factors, and on the operators' experience.

- “**inadequate**”: smearing and/or fixing and/or staining artefacts or blood obscure thyroid cell aggregates
- “**non representative**”: a number of epithelial cells, insufficient for a definitive diagnosis, is collected from the nodule or the sample is not consistent with the target thyroid lesion.

The cytological report should specify if the sample is inadequate or non representative

Nodule structure and rate of TIR 1

Cystic content	No.	Nondiagnostic UG-FNA n (%)
Solid	674	54 (8) ^a
<25% cystic	329	21 (6)
25–50% cystic	142	17 (12)
50–75% cystic	104	26 (25)
>75% cystic	199	71 (36)
Total	1448 ^b	189 (13)

The only predictive factor for TIR 1 is a nodule with a > 50% cystic component

The TIR 1 challenge



Samples obtained from a cystic lesion featuring erythrocytes, necrosis and hemosiderin-laden macrophages without adequate cellularity should be subclassified as **TIR 1 C (Cystic)**

TIR 2 may be adopted in spite of uncomplete sample adequacy/representativeness in case of :

- Abundant and homogeneous colloid aspirated from colloid (“spongiform” at US) nodules or from lesions identified at ultrasonography as cysts;
- Predominant lymphocytic component in a clinically diagnosed Hashimoto thyroiditis
- Cytological pattern consistent with De Quervain thyroiditis

Case 1. Question time: Round 1.



- What is the expected rate of “non diagnostic” FNA ?
- Is FNA repetition useful after a TIR 1 result?

The TIR 1 challenge: facts & figures

- Tir - 1: **5-20%** of FNAs
- Ideally, the inadequate reports (TIR 1) should not exceed **10%** (out of cystic lesions)
- Diagnostic sample after FNA repetition: **47-75%:**
(Chow 2001, Alexander 2002, Piana 2011, Samir 2012)
- Higher percentage of diagnostic result when FNA is performed under US guidance and/or with on-site adequacy assessment.

Comparison of 5-Tiered and 6-Tiered Diagnostic Systems for the Reporting of Thyroid Cytopathology: A Multi-Institutional Study

5-Tiered		6-Tiered (Bethesda)	
TIR 1	7.5	3.0	DCI
TIR 2	83.9	55.4	DCII
TIR 3	4.6	6.7	DCIII (FLUS/AUS)
		23.8	DCIV(FN)
TIR 4	1.1	6.0	DCV
TIR 5	2.9	5.1	DCVI
Total	3962	3724	

Diagnostic categories and clinical management according to the Bethesda system



Diagnostic category	Cytological diagnosis	Risk of malignancy, %	Usual management
I	nondiagnostic or unsatisfactory	1–4	repeat FNA with ultrasound guidance
II	benign	0–3	clinical follow-up
III	AUS/FLUS	5–15	repeat FNA
IV	FNS/SFN	15–30	surgical lobectomy
V	suspicious for malignancy	60–75	near-total thyroidectomy or surgical lobectomy
VI	malignant	97–99	near-total thyroidectomy

Bongiovanni et al. Acta Cytologica 2012; 56:333-339

Diagnostic samples with repeated FNA

Samir et al. (This study)

<i>Cystic vs. solid</i>	<i>Repeat diagnostic FNA (%)</i>
Solid $n = 50$	48
Predominantly solid (<25% cystic) $n = 28$	36
Mixed (25%–75% cystic) $n = 8$	38
Predominantly cystic (>75% cystic) $n = 3$	67
Total $n = 89$	43

Alexander et al. (13)

<i>Cystic vs. solid</i>	<i>Repeat diagnostic FNA (%)</i>
Solid $n = 54$	76
<25% cystic $n = 21$	85
25%–50% cystic $n = 17$	60
50%–75% cystic $n = 26$	54
>75% cystic $n = 71$	48
Total $n = 189$	62

Case 1. Question time: round 2.



- What is the risk of malignancy in a TIR 1 nodule?

Comparison of 5-Tiered and 6-Tiered Diagnostic Systems for the Reporting of Thyroid Cytopathology: A Multi-Institutional Study

5-Tier system			6-Tier system (Bethesda)		
	surgery	malignancy	surgery	malignancy	
TIR 1	5.4	25.0	22.7	32.0	DCI
TIR 2	2.9	3.1	7.7	2.5	DCII
TIR3	56.4	26.5	53.2	14.4	DCIII (FLUS - AUS)
			78.8	32.1	DCIV (FN)
TIR 4	92.9	92.3	81.7	74.9	DCV
TIR 5	94.7	99.1	84.4	99.4	DCVI
Total	9.1	48.9	36.5	41.0	

Bethesda System: a Metanalysis

Cytological diagnosis	All FNAs		All FNAs with histological follow-up			Benign histology		Malignant histology	
	n	% total	n	% total ^a	% category ^b	n	% ^c	n	% ^c
Nondiagnostic	3,271	12.9	530	8.3	16.2	441	83.2	89	16.8
Benign	15,104	59.3	1,563	24.6	10.4	1,505	96.3	58	3.7
AUS/FLUS	2,441	9.6	957	15.0	39.2	805	84.1	152	15.9
FN/SFN	2,571	10.1	1,791	28.2	69.7	1,323	73.9	468	26.1
Suspicious for malignancy	680	2.7	501	7.9	73.7	124	24.8	377	75.2
Malignant	1,378	5.4	1,020	16.0	74.0	14	1.4	1,006	98.6
Total	25,445	100	6,362	100	25.0	4,212	66.2	2,150	33.8

^a Percentage of the 6,362 cases with follow-up. ^b Percentage of cases operated in each DC. ^c Percentage of cases calculated of the total number of operated cases in each category.

Case 1. Question time: round 2.



- Timing of FNA repetition: is it critical for a diagnostic sample?
- Are there any practical tricks to avoid a 2nd non diagnostic FNA?

Case 1. Question time: round 2.



- What to do if FNA repetition reiterates a non diagnostic result?
- Always surgery?

FNA repetition vs. CNB in non diagnostic samples

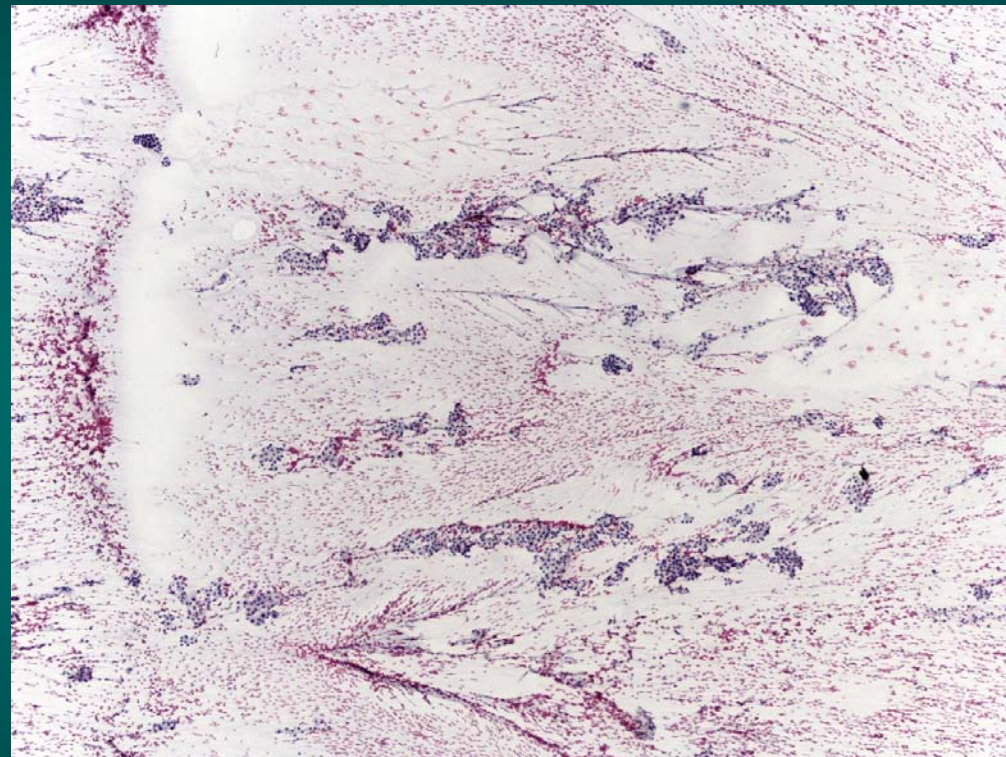
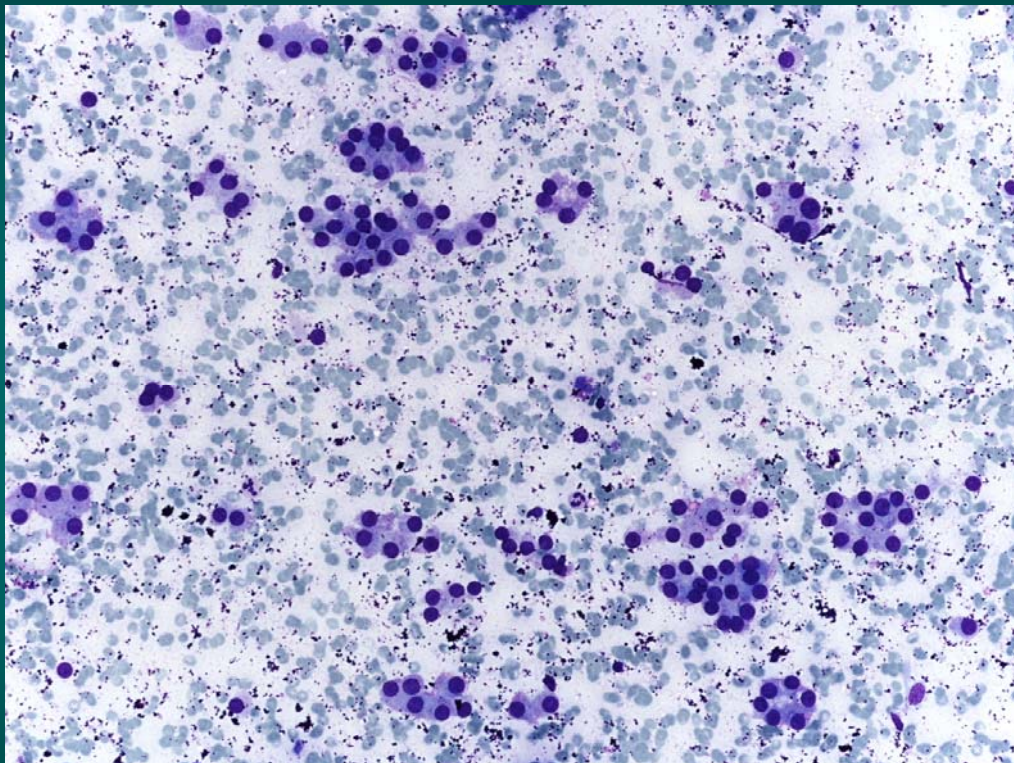


	n. pts	FNA II° (% diagnosis)	CNB (% diagnosis)
Samir et al., 2012	69	52.0	74.0
Na et al., 2012	45	54.7	87.5

US patterns in partially cystic thyroid nodules

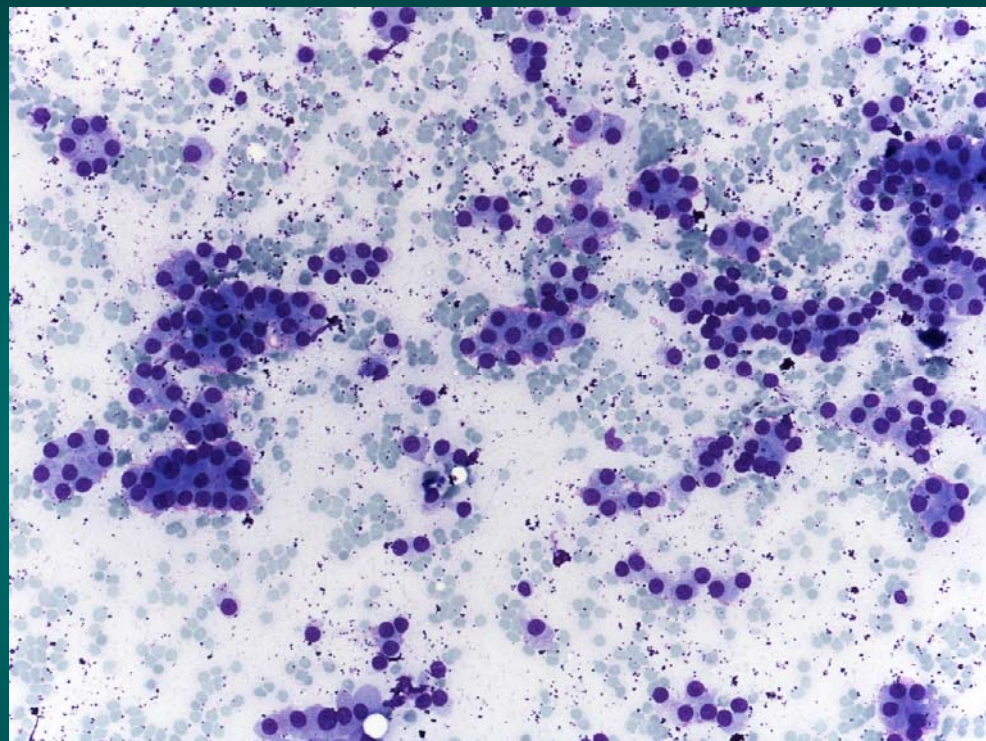
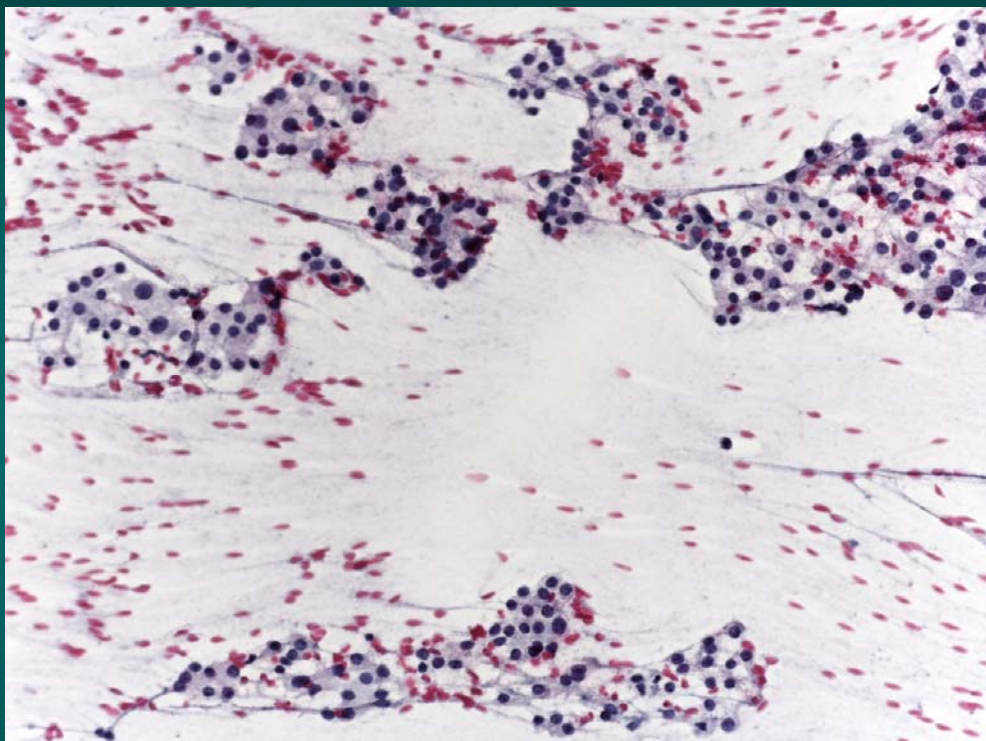
	Benign	Malignant
Configuration	Concentric/eccentric with blunt angle	Eccentric with acute angle
Margins	Smooth, regular	Lobulated, irregular
Vascularity	Peripheral or absent	Centripetal
Solid component	Spongiform, isoechoic	Microcalcification

Clinical Case 1. FNA report (2)



Follicular and microfollicular aggregates of thyroid cells, with mild anisocariosis. Scarce colloid.

Clinical Case 1. FNA report (2)



*Cytological pattern feasible with follicular neoplasm.
SIAPEC Reporting system "TIR 3"*

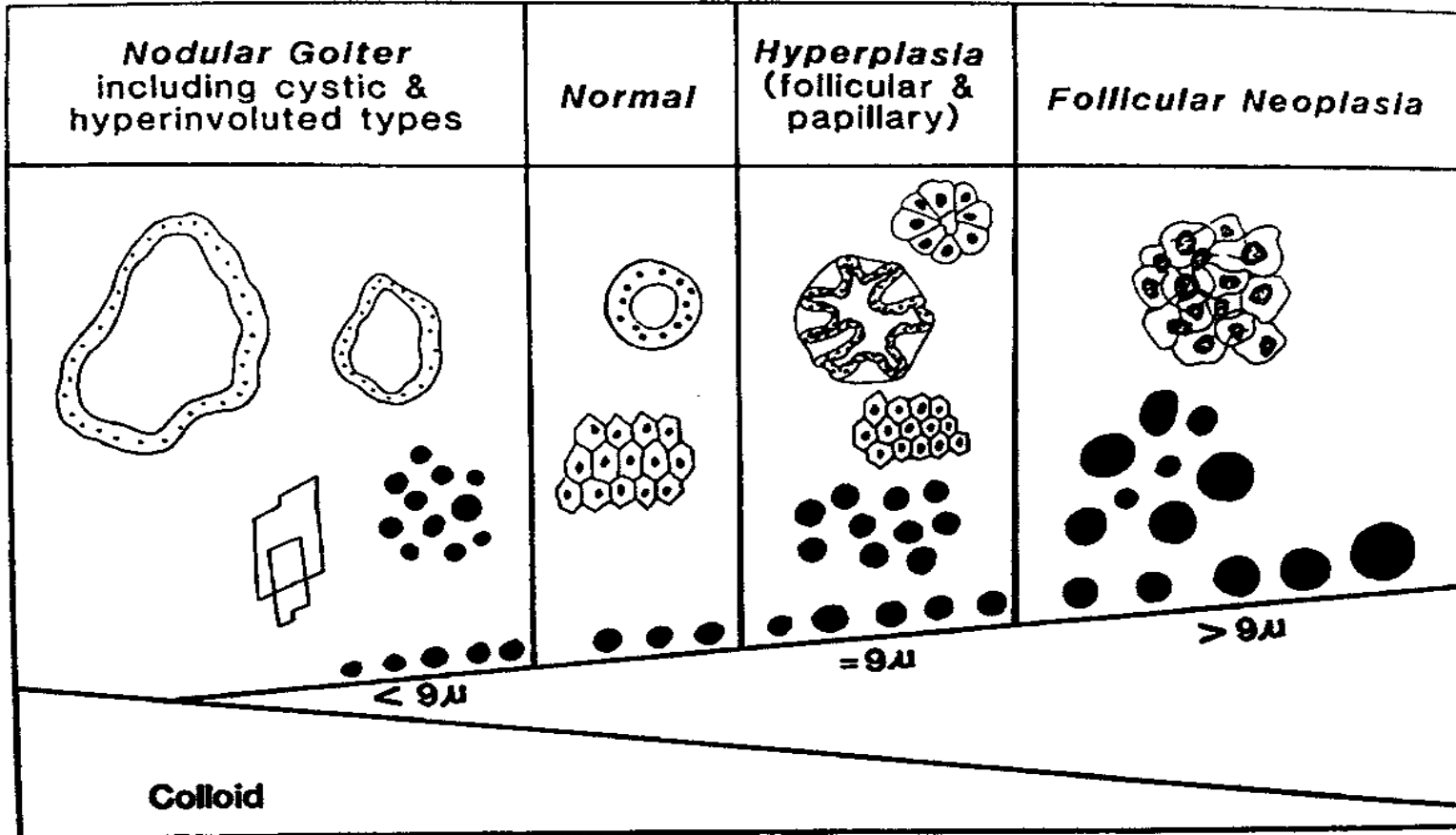
Case 1. Question time: round 3.



- According to the updated Italian Consensus on Thyroid cytology proposal, how should this cytological pattern be classified?

Follicular lesion

morphology: nuclear size/colloid



TIR 3 includes

- **Follicular neoplasms** *i.e. cytologic specimens with high cellularity, predominant microfollicular arrangement and poor/absent colloid component. Samples consisting almost exclusively of Hurthle/oxyphilic cells are included in this category.*
- **Microfollicular/Hurthle cells patterns with moderate colloid amount**, and degenerative/regressive features more likely observed in non neoplastic lesions.
- **Partially compromised specimens** (i.e. low cellularity and/or blood contamination) with **mild cytological or architectural alterations** that may rise some suspicion of malignancy.
- **Cytological alterations** suspicious for malignancy, yet **too scarce to be classified as TIR 4.**

Bethesda System

Atypia of undetermined significance (AUS) and

Follicular lesion of undetermined significance (FLUS) (III)

Follicular Neoplasms (IV)

SIAPEC-SIE-AIT (2013)

- **Tir 3a** Follicular lesions with moderate colloid/minor cytological alterations (**lower risk of malignancy**)
- **Tir 3b** Follicular neoplasms/ suspicious nuclear atypia too scarce to be included in Tir4 (**higher risk of malignancy**)

Case 1. Question time: round 3.



- What is the correlation between cytological and histological diagnosis in follicular neoplasms?
- May CNB help do define the risk of malignancy in follicular neoplasms?

The TIR 3 challenge: definitions



**Indeterminate (TIR 3) lesions
are a moving target...**

Follicular neoplasms: cyto-histological correlations

	Rago et al., 2007	Yang et al., 2007	
Thyroiditis	4 (0.8%)	11 (3.4%)	1.8%
Goiter	52 (10.3%)	53 (16.3%)	12.6%
Follicular /Hürtle cell Adenoma	290 (57.4%)	157 (48.2%)	53.8%
PTC	138 (27.3%)	71 (21.8%)	25.1%
Follicular /Hürtle cell Carcinoma	21 (4.1%)	29 (8.9%)	6.0%
Other malignancy	-	5 (1.5%)	0.6%
Total	505	326	

Results of FNA repetition vs. CNB in “indeterminate” samples

	n. pts	FNA II° (% diagnosis)	CNB (% diagnosis)
Park et al., 2011	142 (FNA) 54 (CNB)	51.4	98.2
Na et al., 2012	104	50.1	73.3
Nasrollah et al., 2012	40	-	47.5

Case 1. Question time: round 4.



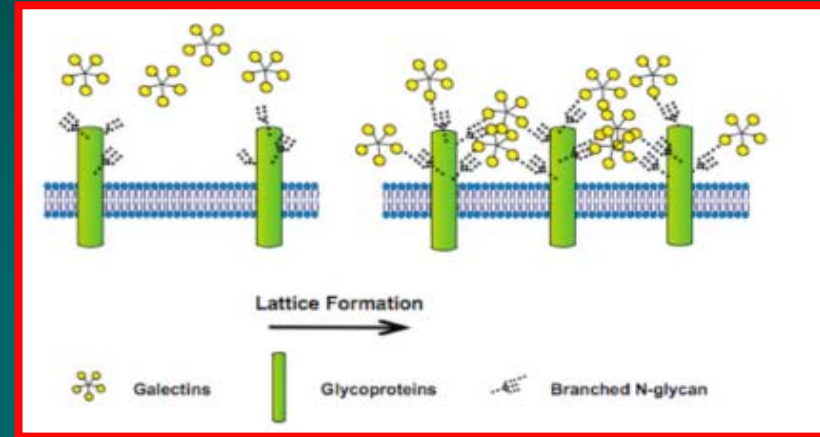
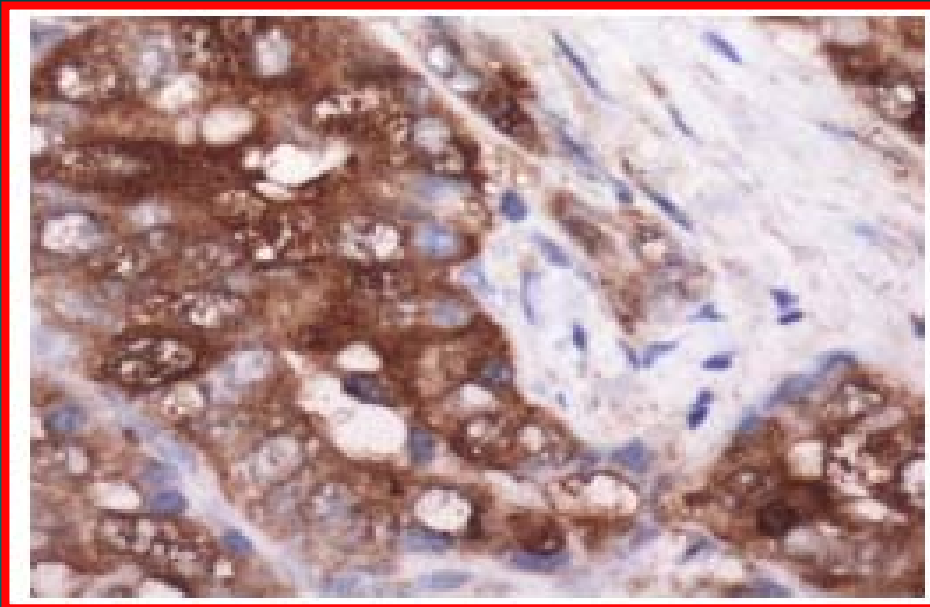
- Molecular markers as “magic bullets” in TIR 3 lesions: clinical science or science-fiction?
- What about BRAF?

Case 1. Question time: round 4.



- How to manage patients with follicular neoplasms: always surgery?

Galectin-3 in the diagnosis of Follicular Thyroid Cancer



Sensitivity 74-100%

Specificity 75-100%

Accuracy 88-100%

Results of molecular testing in Thy-3 lesions (n = 878) from 6 different studies (2010-2012)

		PTC	FTC/HCC	FA/HCA	NH
BRAF	+	34	0	0	0
	-	141	25	235	433
RAS	+	55	7	13	0
	-	83	12	231	378
RET/PTC	+	4	0	0	0
	-	80	19	153	378
PAX8/PPARγ	+	4	-	0	0
	-	94	9	122	348

- Mutation analysis positive in ~60% of malignant and ~2.0% of benign lesions
- High specificity and low sensitivity of mutation analysis in detecting malignant Thy-3 lesions

Clinical features and risk of malignancy in patients with follicular neoplasm

103 patients with FN referred for thyroidectomy,
22 (21%) found to have a malignancy.

Risk of malignancy was significantly higher

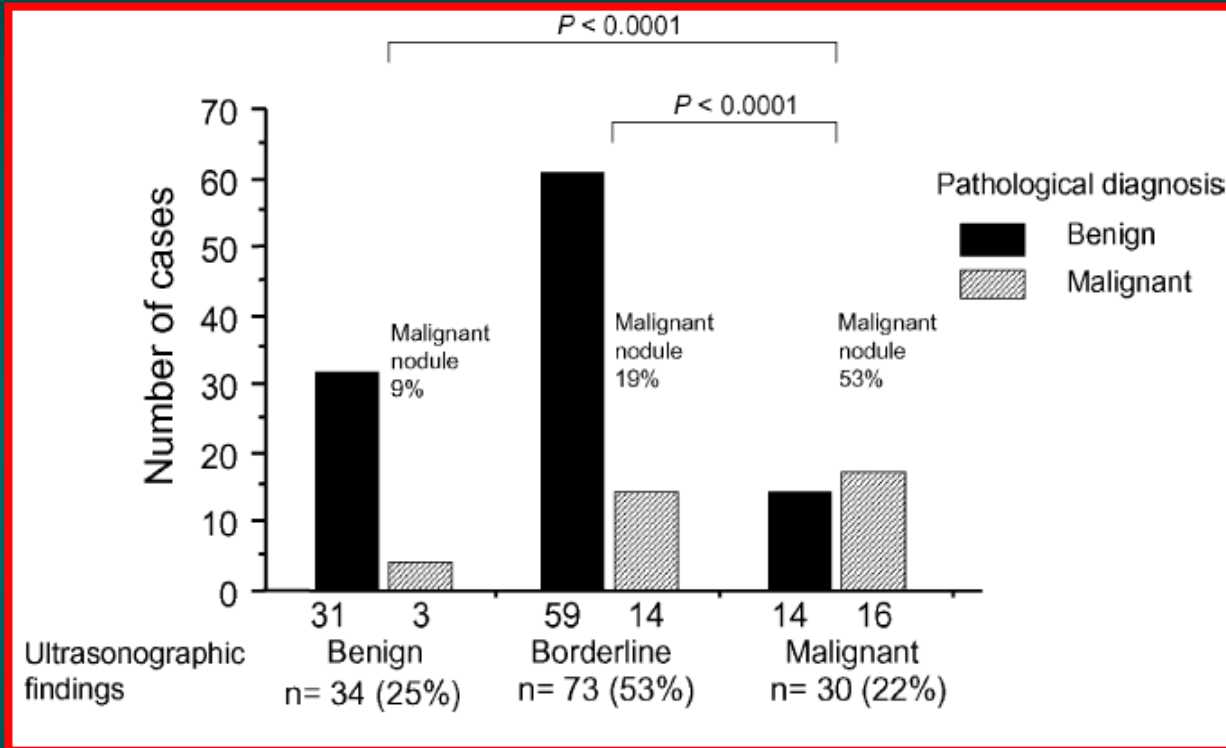
- in males (43% vs. 16% $p = 0.007$),
- in nodule greater than 4 cm (40% vs. 13% $p = 0.03$),
- in solitary nodules (25% vs. 6%, $p = 0.02$).

Risk of malignancy in males with large nodules was nearly 80%, compared with a rate of only 3% in females with small nodules

US findings in a series of 137 follicular neoplasms



Roma,
9-11 novembre 2012



Sensitivity	90.9%
Specificity	29.8%
PPV	29.1%
NPV	91.2%
False negative rate	9.1%
False positive rate	71.2%
Accuracy	44.5%

Benign FN 104 (NH = 72 FA = 32)

Malignant FN 33 (FTC min inv n = 18 FTC wid inv n = 10, PTC n = 5)

53% of malignant-appearing nodules at US corresponding to FTC or follicular variant of PTC at histological examination

Case 1. Question time: round 4.



- Will the Gene classifier systems change the routine clinical practice in the U.S.?

Clinical THYROIDOLOGY

VOLUME 24 • ISSUE 8

AUGUST 2012

A Gene-Expression Classifier on FNA Biopsy of a Thyroid Nodule May Be Helpful to Determine Whether an Indeterminate Nodule Is Benign

Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, Livolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. *N Engl J Med.* June 25, 2012 [Epub ahead of print].

81 FN

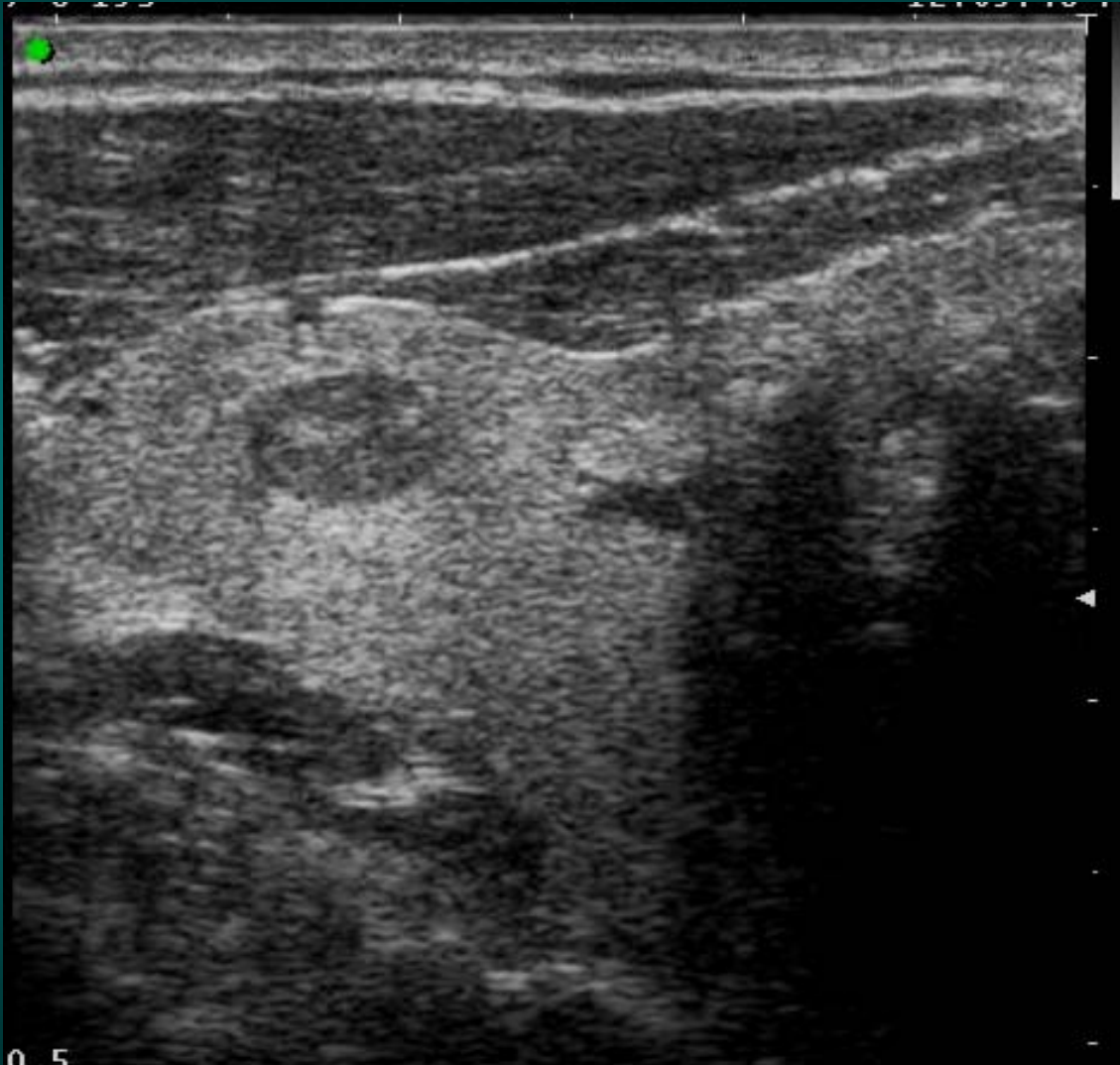
GEC suspicious in

- 18/20 malignant
- 31/61 benign

PPV = 36.7%

NPV = 93.7%

Clinical case 2.

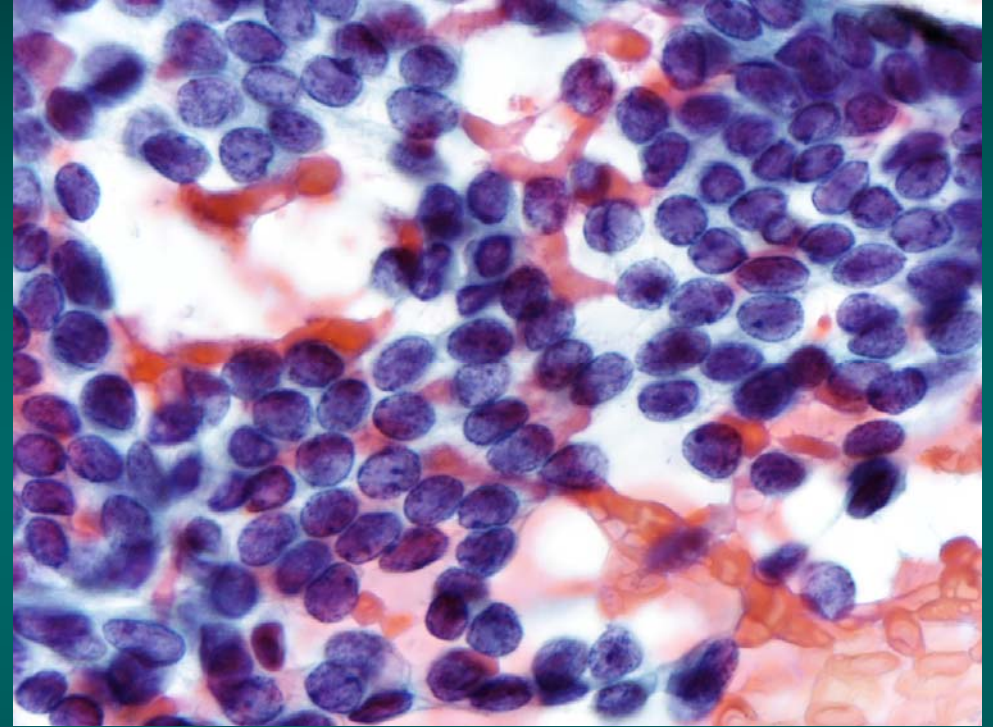
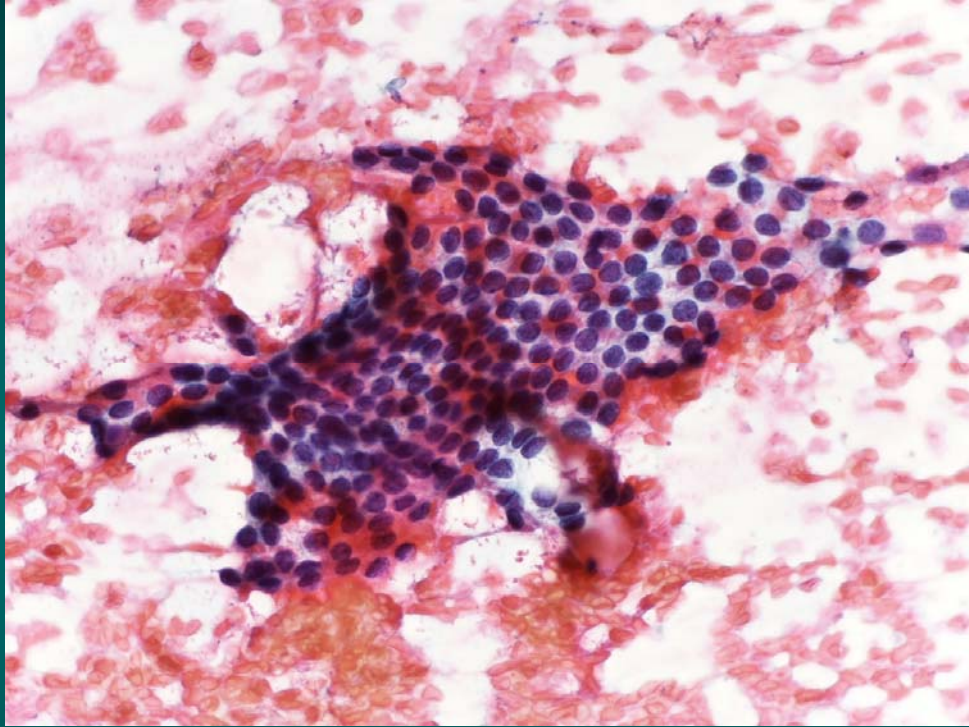


Giulia, age 34

Dermatologist

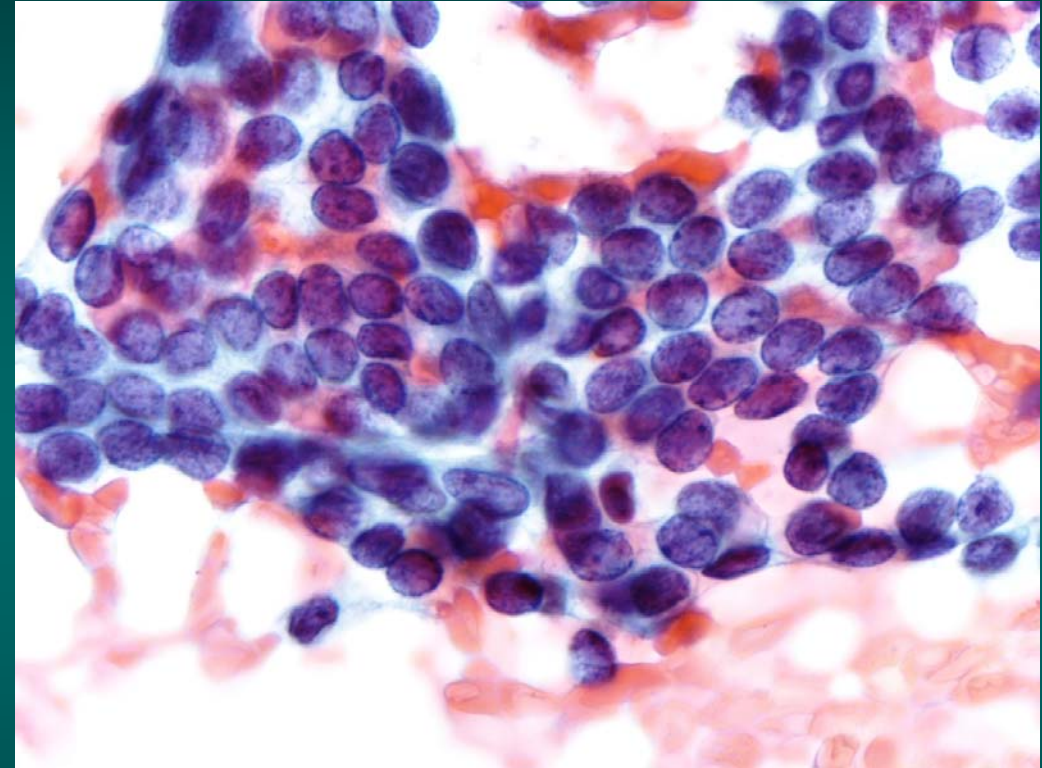
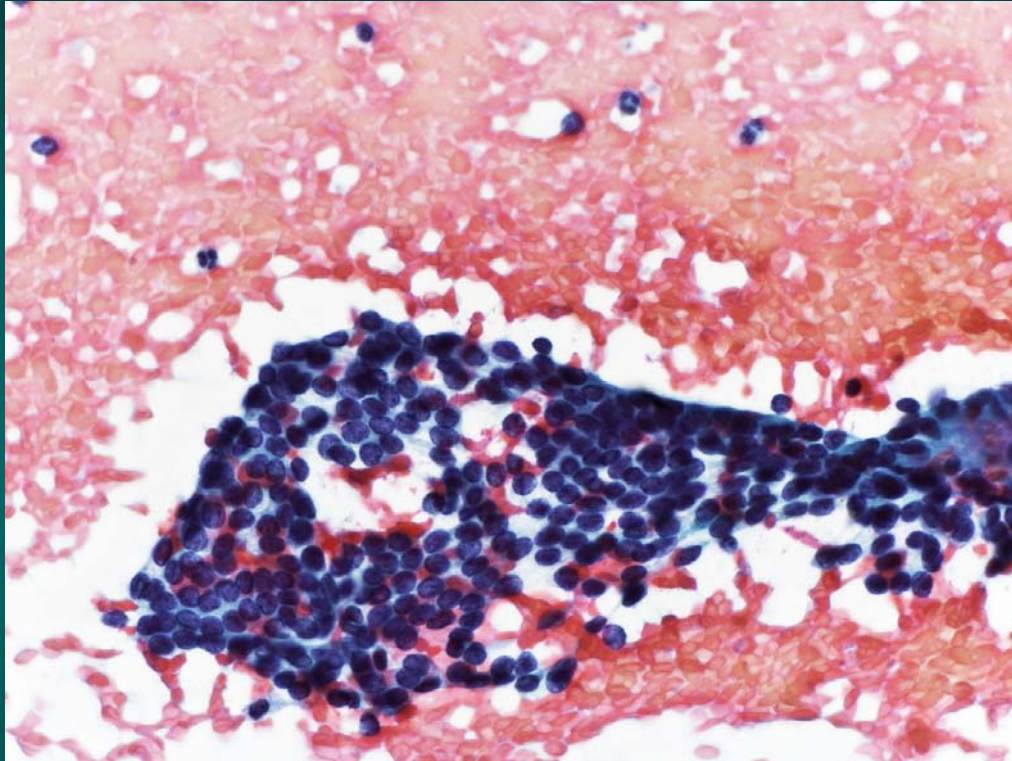
Incidental finding of a
5 mm hypoechoic nodule
with calcifications in the
right thyroid lobe

Clinical case 2: FNA report



US guided FNA: Solid aggregates of epithelial cells with occasional nuclear grooves and indentations. No evidence of nuclear inclusions.

Clinical case 2: FNA report



Cytology: Indeterminate

SIAPEC Reporting system classification: not provided

Question time: round 5.



- How would you classify this lesion?

Bethesda System

Atypia of undetermined significance (AUS) and

Follicular lesion of undetermined significance (FLUS) (III)

Follicular Neoplasms (IV)

SIAPEC-SIE-AIT (2013)

- **Tir 3a** Follicular lesions with moderate colloid/minor cytological alterations (**lower risk of malignancy**)
- **Tir 3b** Follicular neoplasms/ suspicious nuclear atypia too scarce to be included in Tir4 (**higher risk of malignancy**)

Question time: round 5.



- What would you recommend:
 - Repeat FNA ?
 - Molecular markers ?
 - Surgery, anyway ?

FNA repetition in AUS

Repeated Fine-Needle Aspiration Diagnosis Following an Initial Diagnosis of Atypia of Undetermined Significance

Diagnosis	No. (%) of Cases
Benign	139 (48.4)
Atypia of undetermined significance	80 (27.9)
Suspicious for malignancy	26 (9.1)
Suspicious for a follicular/Hürthle cell neoplasm	25 (8.7)
Malignant	5 (1.7)
Nondiagnostic	12 (4.2)
Total	287 (100.0)

**~50% of Benign
at II° FNA**

**~10% of Suspicious or
Malignant at II° FNA**

Surgical Outcome After Initial AUS Diagnosis Stratified by Repeated FNA Diagnosis*

Repeated FNA Diagnosis	No. of Cases	Benign	Malignant
No repeated FNA	90 (45.2)	53 (59)	37 (41)
AUS	51 (25.6)	29 (57)	22 (43)
Benign	7 (3.5)	5 (71)	2 (29)
Nondiagnostic	2 (1.0)	2 (100)	0 (0)
Suspicious for a follicular/Hürthle cell neoplasm	22 (11.1)	13 (59)	9 (41)
Suspicious or positive for malignancy	27 (13.6)	6 (22)	21 (78) [†]
Total	199	108 (54.3)	91 (45.7)

FNA repetition does not apparently influence the rate of malignancy (50-60%) at surgery...

Molecular testing in indeterminate AUS/FLUS and FN lesions

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 <i>RAS</i> (16 PTC,FV) 5 <i>BRAF</i> (4 PTC, 1 PTC,FV) 1 <i>PAX8/PPARg</i> (1 PTC,FV)	3 <i>RAS</i> (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)	

84.9% and 100% rate of malignancy among *RAS*+ and *BRAF*+ lesions, respectively

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 <i>BRAF</i> (1 PTC, 1 PTC,FV) 29 <i>RAS</i> (21 PTC,FV, 5 PTC, 3 FTC) 2 <i>PAX8/PPARg</i> (2 PTC,FV)	5 <i>RAS</i> (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)	

11.4% rate of malignancy among *RAS* and *BRAF* negative lesions

Question time: round 5.

BRAF mutation analysis on Giulia's FNA sample was positive.



- What does the presence of BRAF mutation mean?
- BRAF mutation analysis may change the surgical planning ?

BRAF Mutation analysis in thyroid nodules

Thy-3

		PTC	FTC/HCC	FA/HCA
BRAF	+	34	0	0
	-	141	25	678

Sensitivity = 17.0% Specificity = 100%

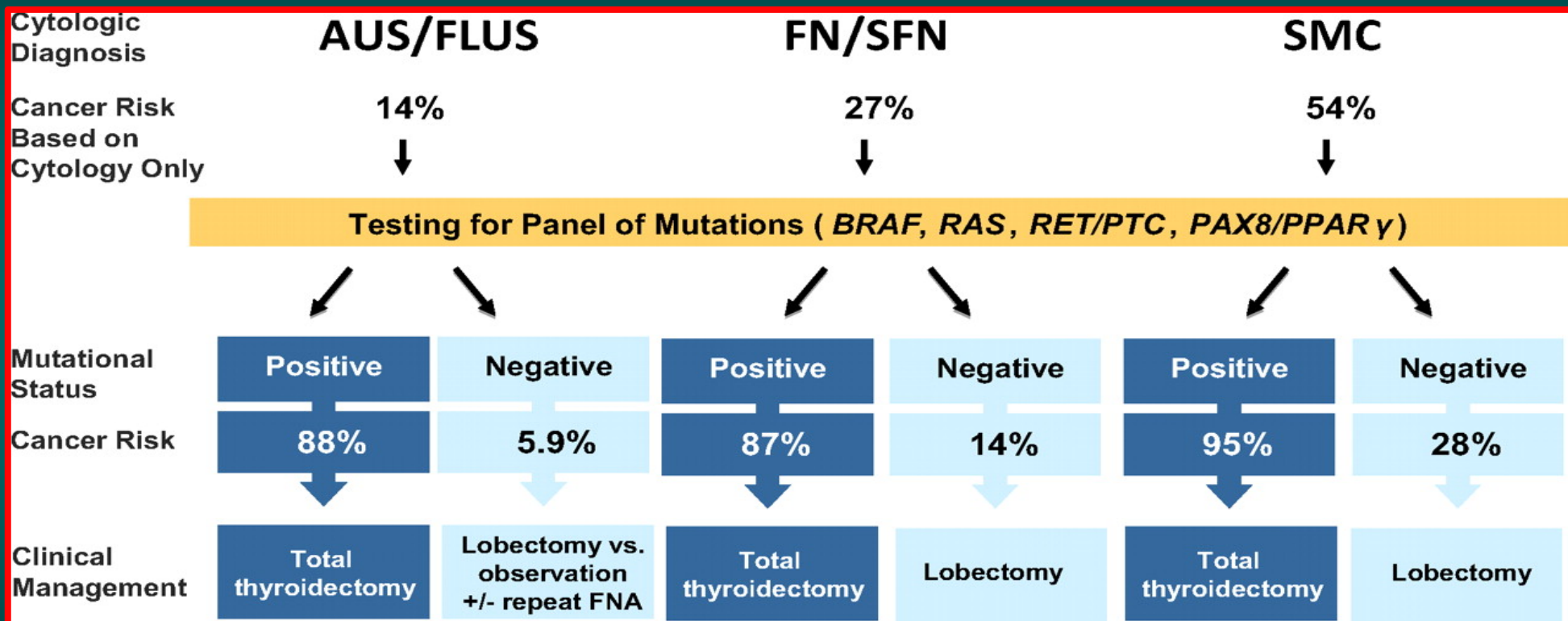
Thy-4/Thy-5

		PTC	FA/Hyperplasia
BRAF	+	111	0
	-	116	35

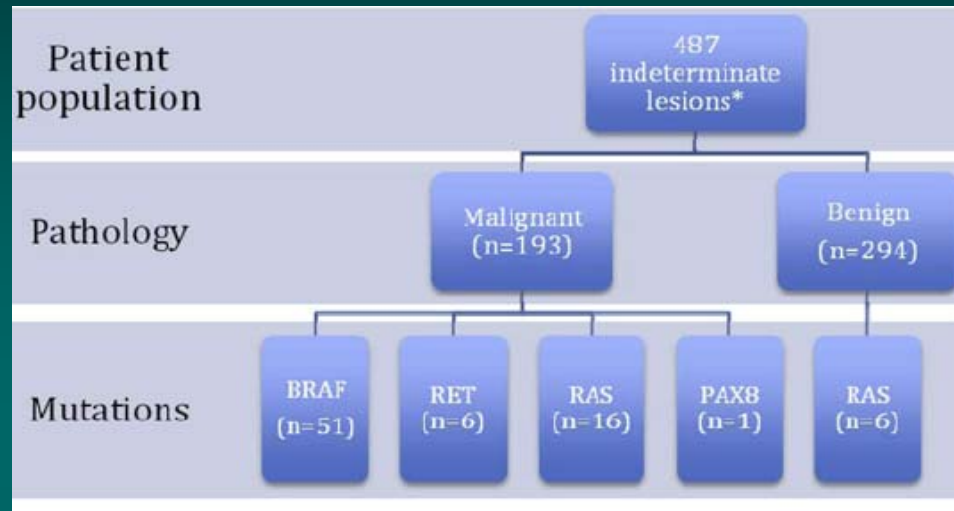
Sensitivity = 48.9% Specificity = 100%

Moses et al. World J Surg. 2010, 34: 2589–2594; Cantara et al. JCE&M, 2010, 95:1365-1369; Ohori et al., Cancer Cytopathol, 2010, 118: 17-23; Nikiforov et al., JCE&M 2011; 96:3390-3397; Canadas Garre et al, Ann Surg, 2012 255:986-992; Zatelli et al. JCE&M, 2012

Algorithm for patients with indeterminate FNA based on the results of mutational analysis.



Risk stratification of indeterminate lesions based on mutation analysis

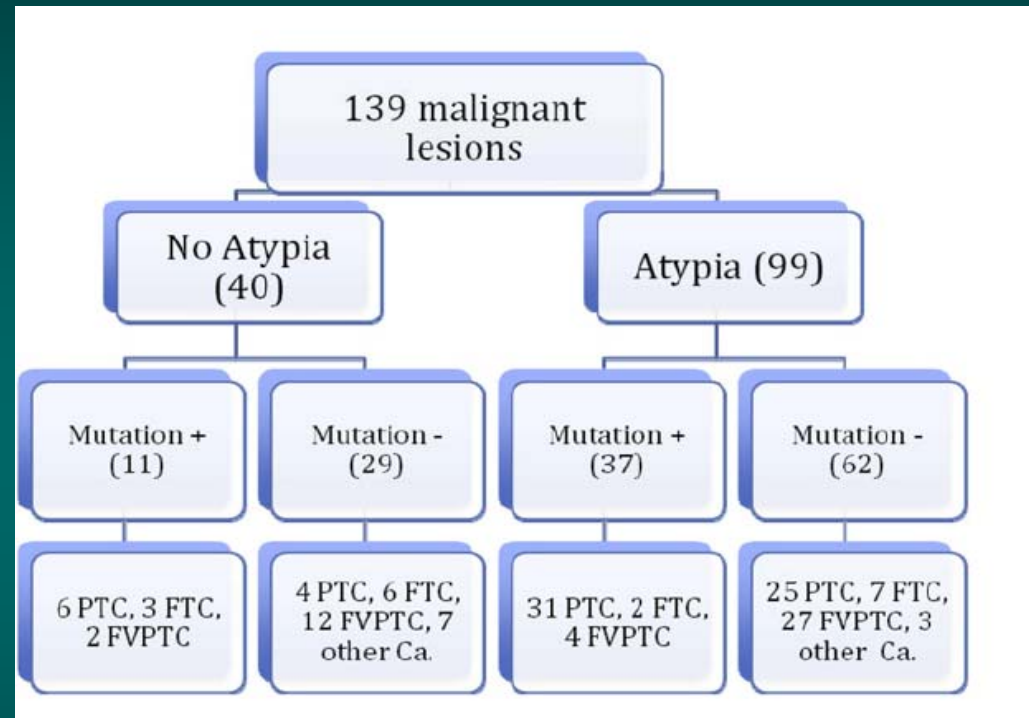


Negative mutation analysis:
risk of malignancy = 22%

Positive mutation analysis
Risk of malignancy = 92%

emithyroidectomy in case of mutation – lesions

total thyroidectomy as initial surgery in case of mutation + lesion



Thy-3 lesions

Clinical and US findings*	Mutation analysis +	Mutation analysis -
Low risk	Lobectomy	Wait and see
High/intermediate risk	Total thyroidectomy (+CND)	Lobectomy

Thy-4 lesions

Clinical and US findings*	Mutation analysis +	Mutation analysis -
Not critical	Total thyroidectomy + CND	Lobectomy + Frozen section

* Include lesion size, US features, controlateral lobe appearance, patient's age/gender

Clinical case 2.



Giulia underwent right emithyroidectomy plus right central neck dissection.

Histology: papillary microcarcinoma (G1 pT1pN0pMx)

And now the take-home messages!

