Performance diagnostica: i dati di letteratura

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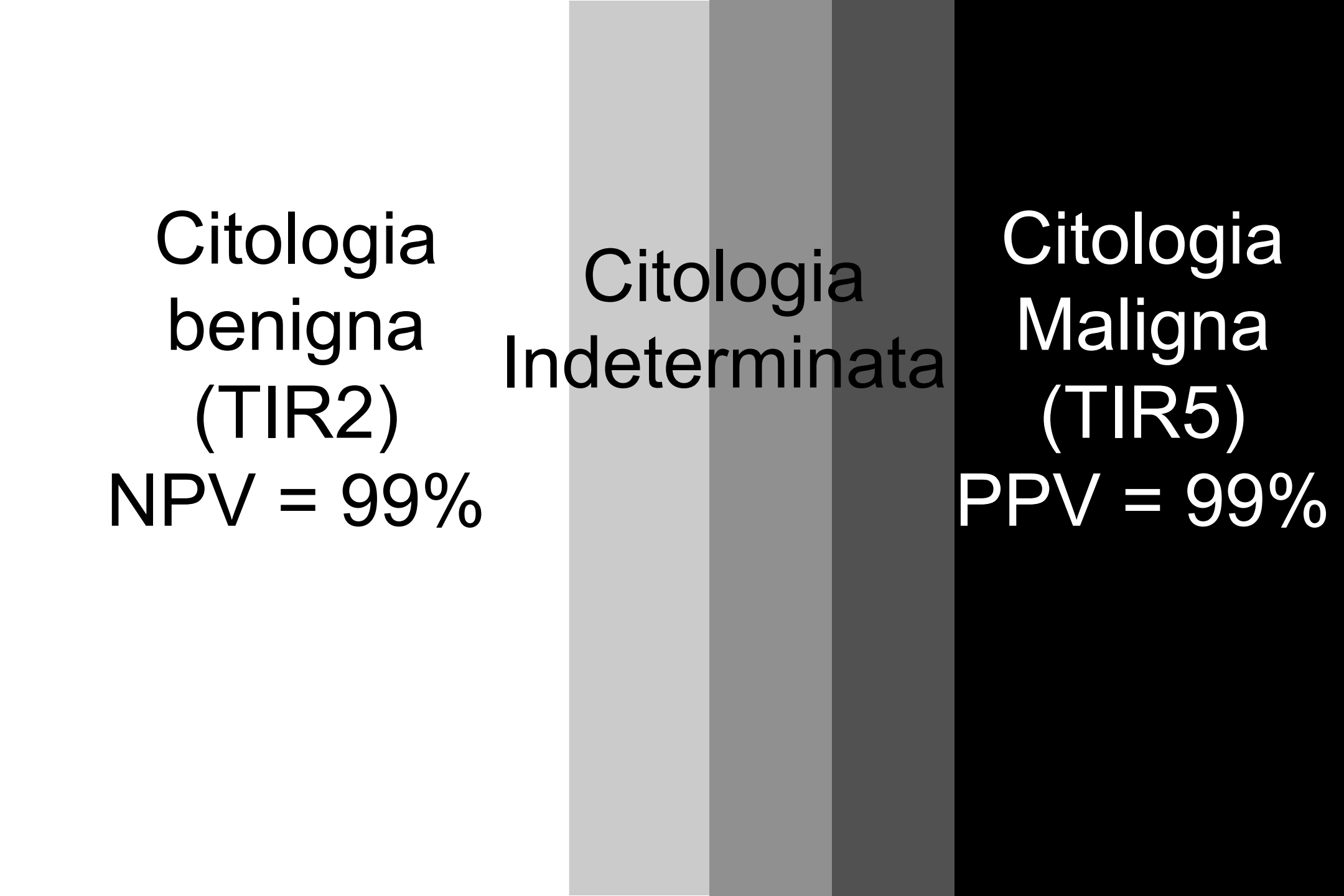
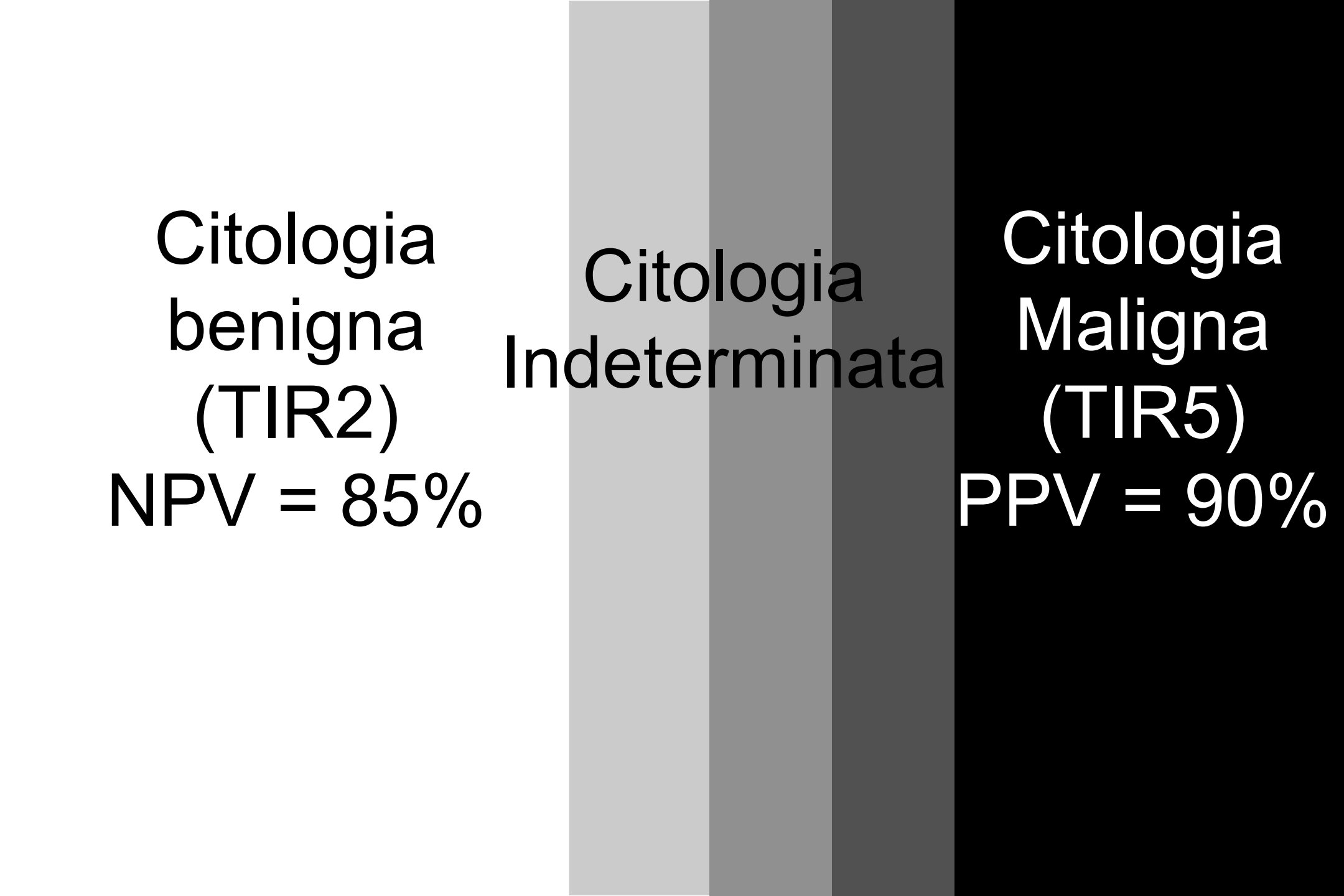

Citologia benigna (TIR2) NPV = 99%
Citologia Indeterminata
Citologia Maligna (TIR5) PPV = 99%

La biologia molecolare nel nodulo "indeterminato"

- Il test di "rule-in" ideale dovrebbe avere un valore predittivo positivo (PPV) pari al TIR5 citologico (~ 99.9%),

- Il test di "rule-out" ideale dovrebbe avere un valore predittivo negativo (NPV) pari al TIR2 citologico (~ 99.9%)


Citologia benigna (TIR2) NPV = 85%
Citologia Indeterminata
Citologia Maligna (TIR5) PPV = 90%

Selecting tests for ruling out or ruling out disease

- If a test is **sensitive**, most of the diseased subjects will be defined as positive after testing . Thus a negative response effectively **rules out** the target disorder.

- With a **specific** test, a positive response makes the presence of the disease more likely. A positive response **rules in** the disorder.

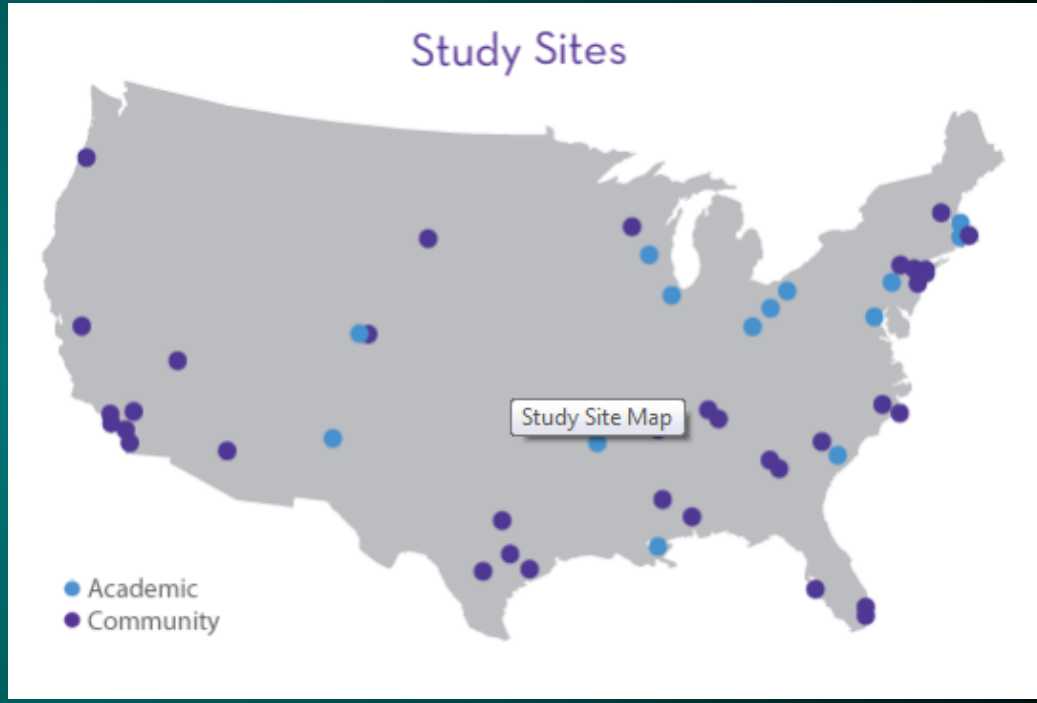
Lee, Int J Epidemiol 1999

Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology

METHODS

mRNA transcripts from 142 genes representing well-known cancer biologic pathways

577 indeterminate, 413 with histopathological diagnosis. Gene-expression classifier (GEC) used to test 265 indeterminate nodules.



RESULTS

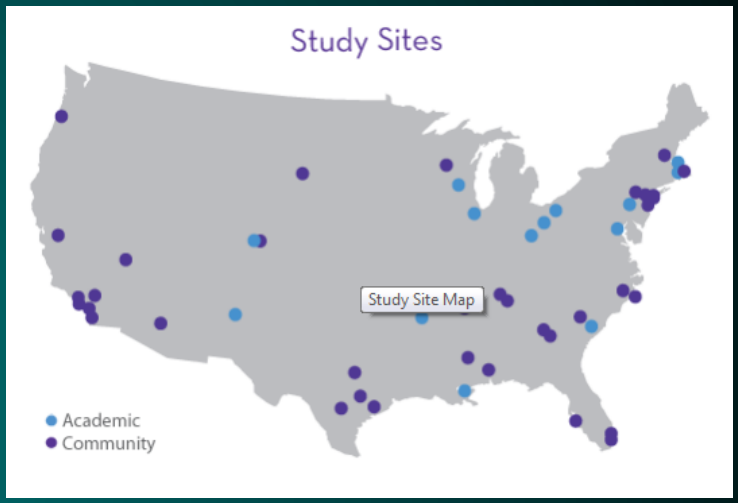
GEC correctly identified 78/85 malignant nodules

Prospective, multicenter double-blind validation study. 49 clinical sites, 3789 patients, and 4812 FNAs.

N Engl J Med 367;8 , 2012

Nodulo indeterminato
Accuratezza del GEC Afirma

92.0% sensitivity
52.0% specificity
NPV = 93.0%
PPV = 47.3%



These data suggest consideration of a more conservative approach for most patients with thyroid nodules that are cytologically indeterminate on fine-needle aspiration and benign according to gene-expression classifier results

Alexander et al., N Engl J Med 367;8 , 2012

Nodulo indeterminato
Accuratezza del GEC Afirma

Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (N=129, 48.7%)

GEC result	Malignant reference standard (N=31)	Benign reference standard (N=98)
Suspicious	28	46
Benign	3	52

Sensitivity, 90% (74–98); specificity, 53% (43–63); PPV, 38% (27–50); NPV, 95% (85–99); prevalence of malignant lesions, 24%

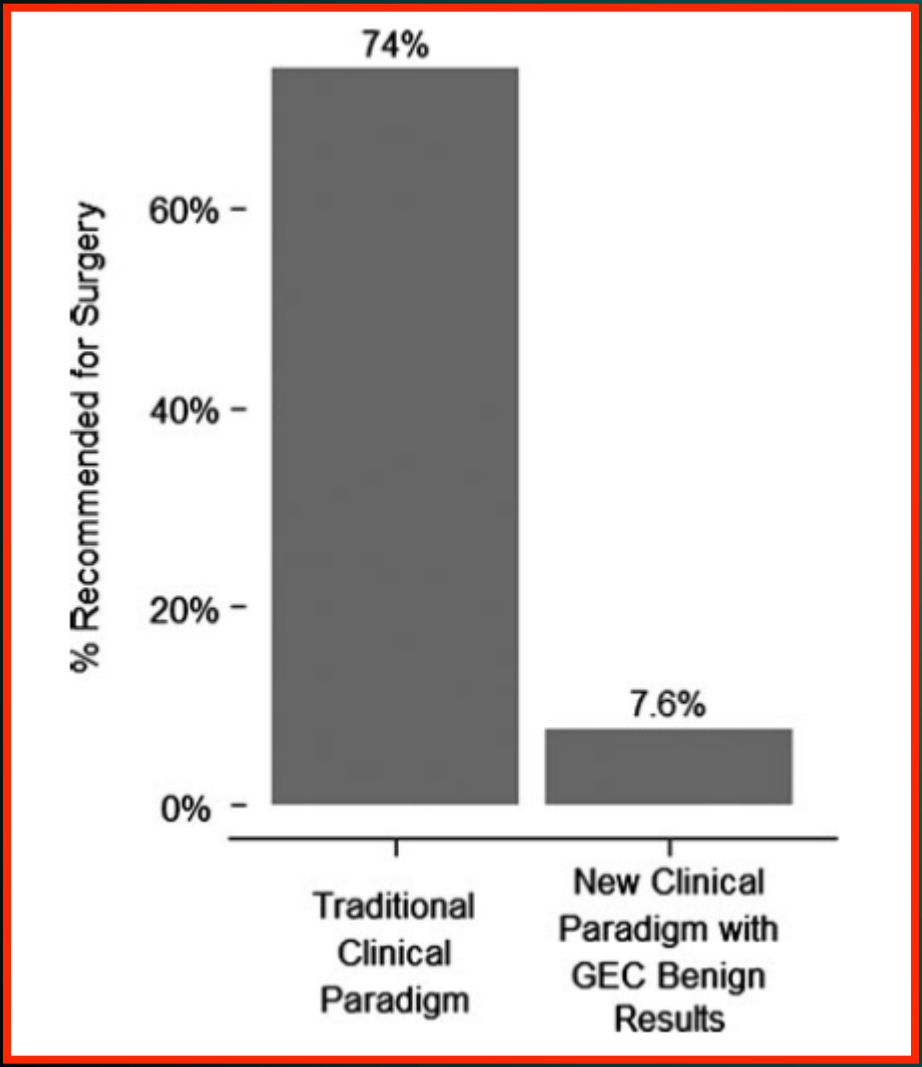
Follicular or Hürthle-Cell Neoplasm or Suspicious for Follicular Neoplasm (N=81, 30.6%)

GEC result	Malignant reference standard (N=20)	Benign reference standard (N=61)
Suspicious	18	31
Benign	2	30

Sensitivity, 90% (68–99); specificity, 49% (36–62); PPV, 37% (23–52); NPV, 94% (79–99); prevalence of malignant lesions, 25%

Alexander et al., N Engl J Med 367;8 , 2012

The Impact of Benign GEC Results on the Decision to Operate on Patients with IndeterminateThyroid Nodules



Cross-sectional cohort study; data contributed retrospectively by 51 endocrinologists at 21 practice sites

The rate of surgery on cytologically indeterminate nodules that were benign by GEC reading did not differ from the historically reported rate of operation on cytologically benign nodules

Duick et al. Thyroid , 22: 996-1001 2012

Analytical Performance Verification of a Molecular Diagnostic for Cytology-Indeterminate Thyroid Nodules

Analytical sensitivity, analytical specificity, robustness, and quality control of the GEC were successfully verified, indicating its suitability for clinical use.

Cost of the test about 3200 $

Walsh et al. JCEM, 2012 2012 Dec;97(12):E2297-306

Cost-Effectiveness of a Novel Molecular Test for Cytologically Indeterminate Thyroid Nodules

- Use of the molecular test resulted in 74% fewer surgeries for benign nodules with no greater number of untreated cancers. Over 5 yr, mean discounted cost estimates were $12,172 for current practice and $10,719 with the molecular test.
- Compared with current practice based on cytological findings alone, use of this test may result in lower overall costs and modestly improved quality of life for patients with indeterminate thyroid nodules

J Clin Endocrinol Metab 96: E1719–E1726, 2011

An Independent Study of a Gene Expression Classifier (Afirma™) in the Evaluation of Cytologically Indeterminate Thyroid Nodules

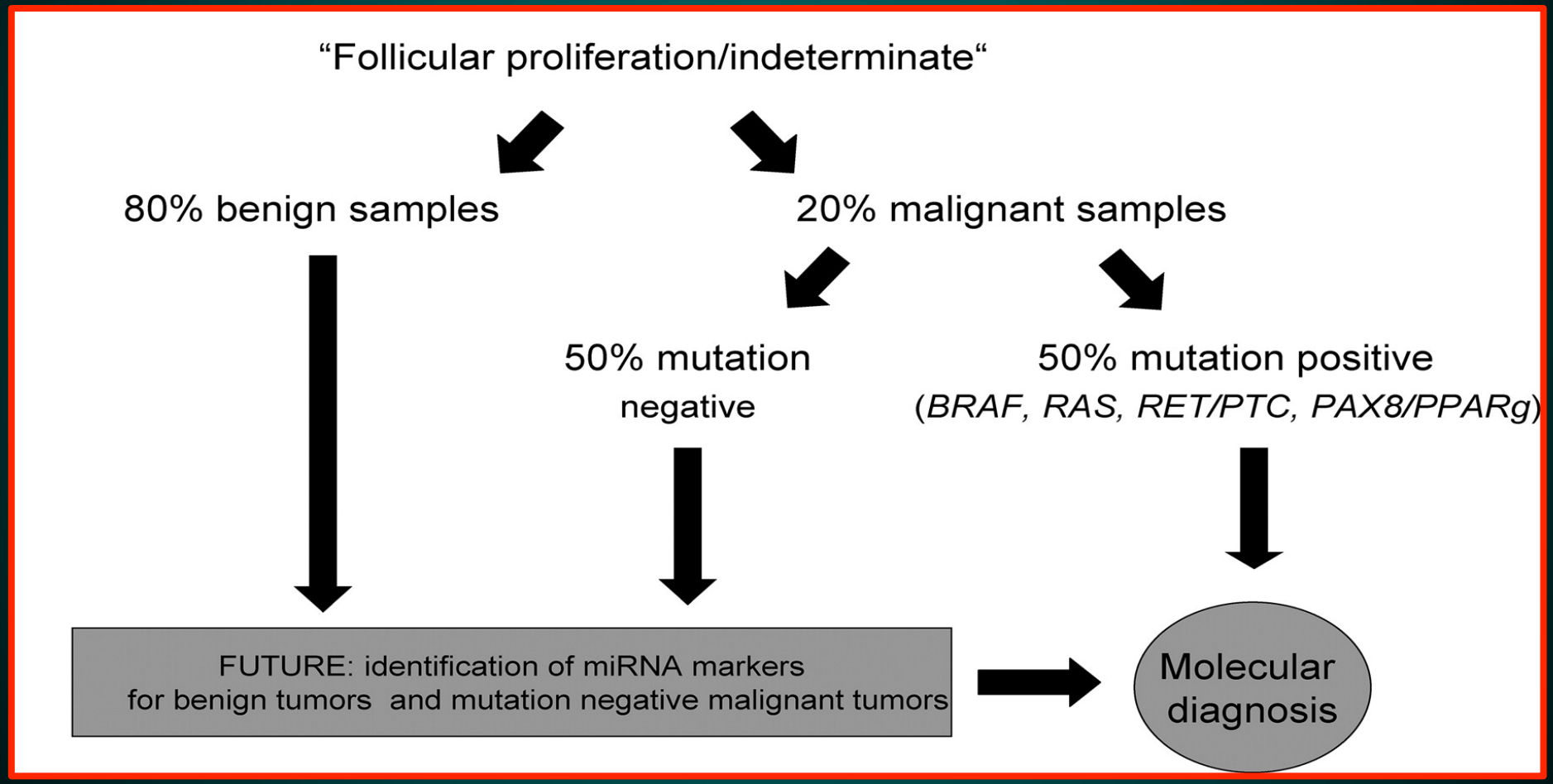
Sensitivity 83%; Specificity 10%; NPV 75%; PPV 16%

Performance across the Entire Data set of indeterminate samples (SFN +SHCN + Atypia/FLUS)			
GEC RESULTS	HISTOPATHOLOGY		
	MALIGNANT	BENIGN	TOTAL
SUSPICIOUS	5	27	32
BENIGN	1	3	4
TOTAL	6	30	36

Positive Predictive Value of the Afirma GEC is lower than previously reported, and call into question the performance of the test when applied in the context of specialized academic cytopathology.

McIver et al. JCE&M, 2014

led value of molecular methods for follicular proliferation/indeterminate FNAB



Ferraz C et al. JCEM 2011;96:2016-2026

he alternative commercially available approach

Asuragen

miR*Inform* Thyroid

Asuragen Clinical Laboratory
2150 Woodward St., Ste. 100
Austin, TX 78744
(877) 777-1874
ClinicalLabSupport@Asuragen.com

XX/XX/2011

Patient Name: Doe, Jane
Order ID: X1234567
MRN: MRN12345676
Attending Provider: William Smith, MD

DOB: 01/01/1961
Lab ID: L987654
Specimen ID: SPEC9876
Date Collected: 11/28/2010

Sex: Female
Type of Sample: FNA
Date Received: 12/01/2010

Mutation	Result	Mutation	Result
BRAF p.V600E (GTG>GAG)	+	*KRAS p.G12S (GGT>AGT)*	-
NRAS p.Q61R (CAA>CGA)	-	*KRAS p.G12R (GGT>CGT)*	-
NRAS p.Q61K (CAA>AAA)	-	*KRAS p.G12V (GGT>GTT)*	-
NRAS p.Q61L (CAA>CTA)	-	*KRAS p.G13D (GGC>GAC)*	-
HRAS p.G12V (GGC>GTC)	-	*KRAS p.G12D (GGT>GAT)*	-
HRAS p.Q61L (CAG>AAG)	-	*RNA Translocations*	
HRAS p.Q61R (CAG>CGG)	-	*RET/PTC1 TRANSLOCATION*	-
KRAS p.G12A (GGT>GCT)	-	*RET/PTC3 TRANSLOCATION*	-
KRAS p.G12C (GGT>TGT)	-	*PAX8/PPARγ TRANSLOCATION*	-

Indeterminate nodules (Thy-3)
Accuracy of Molecular Tests

	All 4 studies*
PTC	34
FTC	27
fvPTC	80
Adenoma	267
Hyperplasia	351
total	706

Sens	54.2%
Spec	95.8%
PPV	74.7%
NPV	90.1%
Accuracy	88%

Cantara et al., JCEM 2010, 95: 1365-1369
Ohori et al., Cancer Cytopathol 2010,
Nikiforov Y E et al. JCEM 2011;96:3390-3397
Eszlinger et al., Thyroid 2013 (

Gestione del nodulo indeterminato (TIR 3)

1000 pazienti sottoposti a FNA

Risultato TIR 3 nel 10% dei casi

Senza Analisi Molecolare

N=100

Ripetizione FNA

N=15

TIR4/5 n = 5
TIR1/2 n = 10

TIR 3 confermato
n = 85

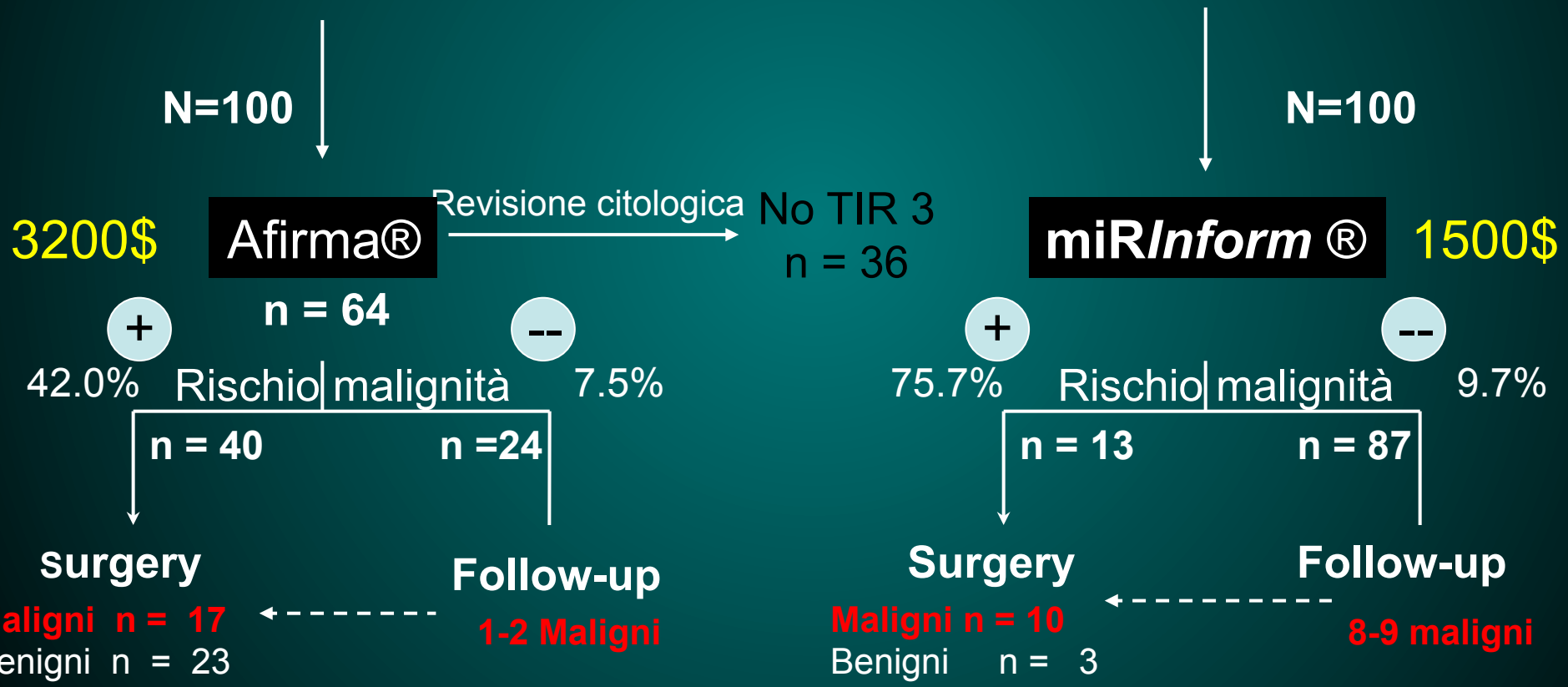
Chirurgia (70%)	**Follow-up (30%)**
n = 60	**n = 25**
Maligni n = 16	**Maligni 7-8?**
Benigni n = 47	

Con Analisi Molecolare

Gestione del nodulo indeterminato (TIR 3)

1000 pazienti che sottoposti a FNA

Risultato TIR 3 nel 10% dei casi



hyroSeq v2 Next-Generation Sequencing in Follicular Neoplasm Cytology

- 143 consecutive FN/SFN with known surgical outcomes
- Analysed with the targeted ThyroSeq v2 NGS panel (point mutations in 13 genes and 42 types of gene fusions that occur in thyroid cancer).
- In the entire cohort, histologic analysis revealed 104 benign nodules and 39 malignant nodules. The most common point mutations involved NRAS, KRAS, TERT and TSHR gene. The identified fusions involved THADA, PPARG and NTRK3 gene.

Nikiforov et al. Cancer 2014;120:3627-34

ThyroSeq v2 Next-Generation Sequencing in Follicular Neoplasm Cytology

Alteration	Positive	Unique Diagnostic Events	Cancer Identified at Surgery (Cancer Risk, %)	Negative Because of Low Level
Point mutations				
NRAS	16	13	13 (81)	2
KRAS	6	6	5 (83)	4
HRAS	2	2	2 (100)	0
TERT	4	2	4 (100)	0
TSHR	3	3	1 (33)	1
BRAF V600E	1	1	1 (100)	0
BRAF K601E	1	1	0 (0)	0
TP53	1	0	1 (100)	0
PIK3CA	1	0	1 (100)	0
Gene fusions				
THADA	5	5	5 (100)	0
PPARG	4	4	4 (100)	0
NTRK3	2	2	2 (100)	0

Nikiforov et al. Cancer 2014;120:3627-34

ThyroSeq v2 performance

- 90% sensitivity
- 93% specificity
- 83% PPV
- 96% NPV
- 92% accuracy

The current results indicate that comprehensive genotyping of thyroid nodules using a broad NGS panel provides a highly accurate diagnosis for nodules with FN/SFN cytology and should facilitate the optimal management of these patients

Nikiforov et al. Cancer 2014;120:3627-34

Indeterminate nodules (Thy-3) Positive Predictive Value

	Cantara et al., 2010*	Nikiforov et al., 2011	Ohori et al., 2010	Eszlinger et al., 2013
PTC	7	15	11	2
FTC	0	9	0	18
fvPTC	0	69	9	2
Adenoma	26	107	15	119
Hyperplasia	8	261	82	-
total	41	461	117	141

* Pax8PPARγ n.p.

PPV (%)				
FA as TN/FP	85.7	87.3	100	77.2
FA as TP/FN	100	100	100	100

Indeterminate nodules (Thy-3) Negative Predictive Value

	Cantara et al., 2010*	Nikiforov et al., 2011	Ohori et al., 2010	Eszlinger et al., 2013
PTC	7	15	11	2
FTC	0	9	0	18
fvPTC	0	69	9	2
Adenoma	26	107	15	119
Hyperplasia	8	261	82	-
total	41	461	117	141

* Pax8PPARγ n.p.

NPV (%)				
FA as TN/FP	97.0	90.4	92.4	89.4
FA as TP/FN	23.5	65.6	78.1	89.4.

	Cantara et al., 2010[1] (10)		Nikiforov et al., 2009[1] (12)		Nikiforov et al., 2011 (13)		Ohori et al., 2010 (14)		this study	
indeterminates	*N*		*N*		*N*		*N*		*N*	
PTCs	53		13?		33		11?		2	
FTCs	0		4		11		0		19	
fvPTCs	0		4?		77		9?		4	
FAs	42		31		392		97		139	
sum	*95*		*52*		*513*		*117*		*164*	
indeterminates (+ suspicious[1])	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*
mFNA **vs.** histology[2]	81	98	71	100	61	98	60	100	18	86
mFNA **vs.** histology[3]	86	100	71	100	64	100	60	100	54	100
indeterminates (FTC/FA)	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*
mFNA **vs.** histology[2]	N/A	N/A	50	100	45	98	N/A	N/A	22	84
mFNA **vs.** histology[3]	N/A	N/A	50	100	64	100	N/A	N/A	60	100
malignant + indeterminate + (suspicious +) benign	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*	*sensitivity*	*specificity*
cytology **vs.** histology	59	95	44	100	N/A	N/A	N/A	N/A	67	100
cytology + mFNA **vs.** histology[2]	89.7	94.9	80	99.7	N/A	N/A	N/A	N/A	75	90
cytology + mFNA **vs.** histology[3]	90.5	98.7	84	100	N/A	N/A	N/A	N/A	80	100

the sensitivities and specificities of the studies are comparable when reducing the indeterminate sample set to FTCs and FAs.

Eszlinger et al., Thyroid 2014 Feb;24(2):305-13

Impact of molecular screening for point mutations and rearrangements in routine air-dried FNA samples of thyroid nodules.

Methods

RNA and DNA from 310 FNAs (164 indeterminate, 57 malignant, 89 benign) and corresponding formalin-fixed paraffin-embedded tissue (156 FA, 32 FTCs, 44 PTC 9 fvPTC and 69 goiters).

Results

In the indeterminate group molecular FNA screening increased the sensitivity from 67% (cytology alone) to 75% (cytology plus molecular screening).

Eszlinger et al., Thyroid 2014 Feb;24(2):305-13

Molecular Testing of Thyroid FNAs Improves Presurgical Diagnosis of Minimally Invasive Follicular Thyroid Carcinomas

347 routine air-dried FNA smears with available histology.

Cytology

8 Thy, 106 Thy-2, 163 Thy-3, 39 Thy-4, 31 Thy-5

Histology

64 cPTC , 2 tcPTC, 21 fvPTC, 21 FTC, 5 HCC,
70 FA 24 HCA,137 goiters

Eszlinger, Piana et al., Thyroid 2015

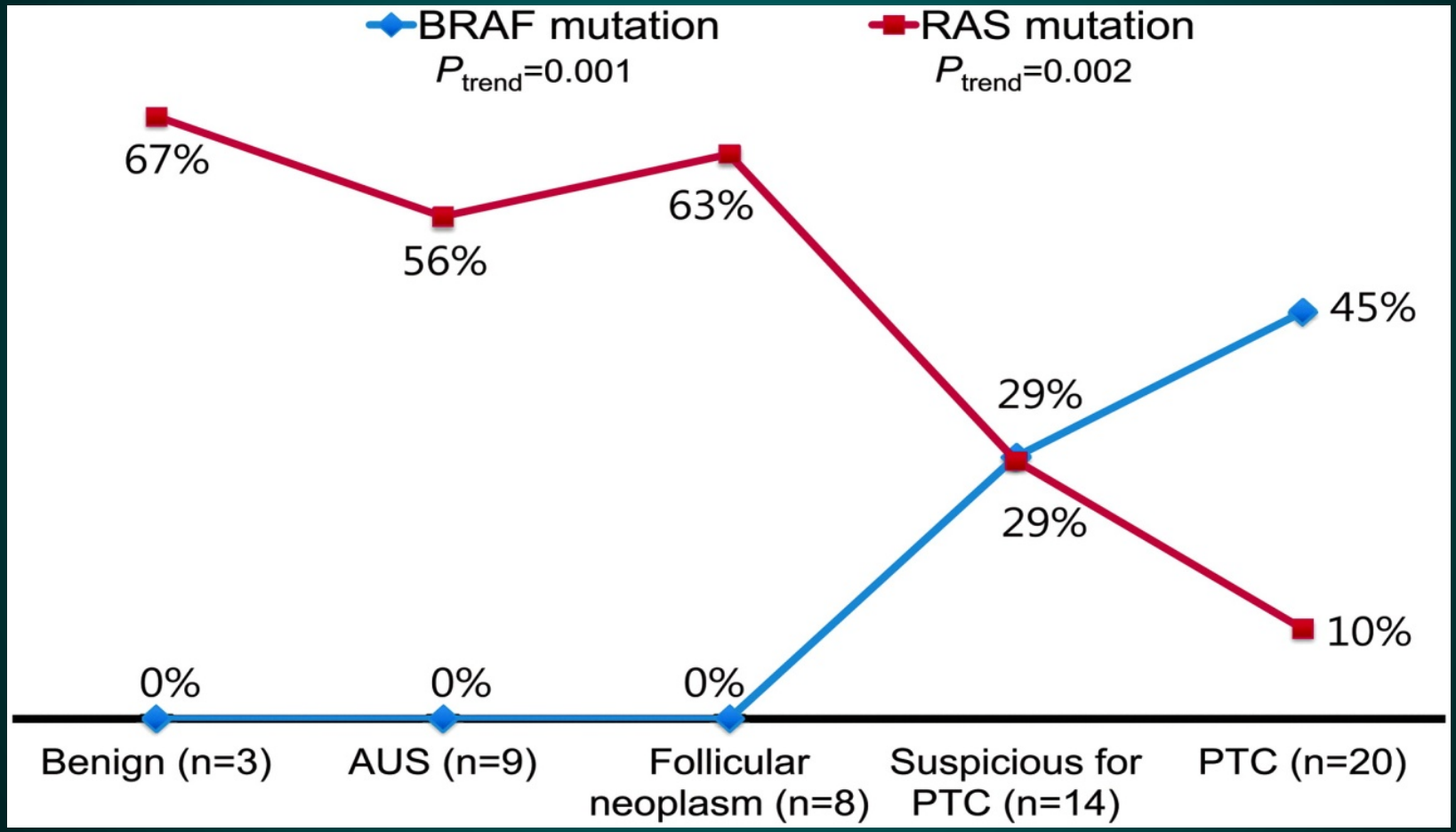
Molecular Testing of Thyroid FNAs Improves Presurgical Diagnosis of Minimally Invasive Follicular Thyroid Carcinomas

Cytology class	*Pre-ROM*	*Post-ROM for mutation-positive samples*	*Post-ROM for mutation-negative samples*
Thy3	28%	71%	18%
Thy4	97%	100%	90%
Thy3 + Thy4	41%	85%	23%
Thy5	100%	100%	100%

49% of the carcinomas identified by molecular testing in the group of follicular lesions (sensitivity increased from 60% to 80% compared to cytologic FNA evaluation alone).

Eszlinger, Piana et al., Thyroid 2015

Accuracy of molecular tests may vary across different Thy-3 series



Lee et al., Thyroid 2013

BRAF Mutation analysis

Tir-3

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

Sensibilità = 17.0% Specificità = 100%

Tir-4/Tir-5

		PTC	FA/Hyperplasia
BRAF	+	111	0
	-	116	35

Sensibilità = 48.9% Specificità = 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

Relevance of BRAFV600E Mutation Testing Versus RAS Point Mutations and RET/PTC Rearrangements Evaluation in the Diagnosis of Thyroid Cancer

TABLE 4. (B) DIAGNOSTIC VALUE OF CYTOLOGY COMBINED WITH GENETIC ANALYSES IN ALL 940 SAMPLES

	Cytology combined with			
	BRAF	RAS	RET/PTC	*All genetic analyses*
PPV	100	76.3	61.1	66.7
NPV	72.6	45.6	38.1	51.9
Sensitivity	76.4	40.3	45.8	82.2
Specificity	100	80	53.3	31.8
Accuracy	85.5	55.6	48.7	63.2

BRAF mutation testing provided the best contribution to cancer diagnosis, whereas RAS and RET/PTC analysis did not further increase diagnostic sensitivity for thyroid cancer.

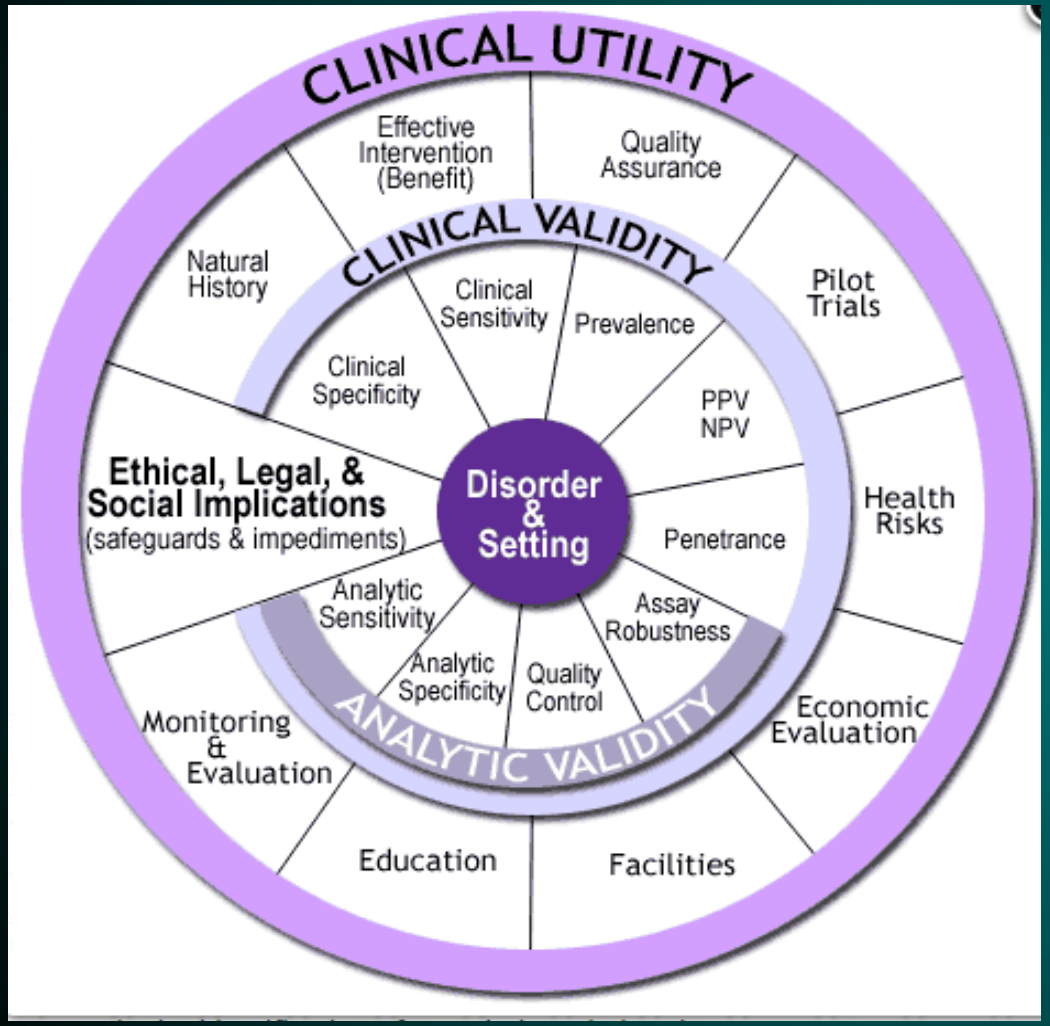
Rossi et al. THYROID Volume 25, Number 2, 2015

AUS/FLUS Cytology

- BRAF : elevatissima specificità, bassa sensibilità.

- L'impiego di un panel di mutazioni (BRAF, NRAS, HRAS, KRAS, RET/PTC1, RET/PTC3, PAX8/PPARγ) alza la sensibilità al 63-80% mantenendo una specificità ancora ~ 100% specificity (le mutazioni di RAS hanno un 15-20% di probabilità di corrispondere ad una lesione benigna

The "ACCE" model system



4 basic criteria for evaluating a genetic test:

Analytical validity,
Clinical validity,
Clinical utility,
Ethical, legal and social issues

Standard set of 44 targeted questions that address disorder, testing, and clinical scenarios, as well as ACCE issues

Journal of Public Health 2007; 29: 246–250

La validazione di un marcatore molecolare si basa su :

- a) **Validità analitica** (accuratezza e riproducibilità del test nel valutare l'evento molecolare)
- b) **Validità clinica** (la performance del test in una determinata popolazione per valutare una determinata condizione patologica)
- c) **Utilità clinica** (capacità del test di incidere su outcome clinico e decision-making).

Validità Analitica

- E’ intrinseca alla metodica e ne indica la capacità di misurare accuratamente e con sufficiente affidabilità una determinata mutazione
- Esprime la performance del test in laboratorio e non nel setting clinico.
- Le tecnologie basate sull’analisi del DNA raggiungono sensibilità e specificità vicine al 100%.

Validità Clinica

- Indica la capacità di un test genetico di individuare o predire la presenza (o l'assenza) di un determinato fenotipo o di una determinata malattia.
- Nella maggior parte dei casi, non essendo note tutte le possibili mutazioni, la sensibilità del test non raggiunge mai il 100%.
- Ciò significa che un test genetico con validità analitica pari al 100% avrà comunque dei limiti di accuratezza nel setting clinico

Utilità Clinica

Indica la probabilità che l'impiego del test migliori l'outcome Clinico. Le domande a cui rispondere:

- Quale è lo scopo del test? Il risultato modifica gestione clinica o prognosi del paziente? Ci sono altre metodologie che ottengono risultati analoghi a quelli del test genetico?

- Quale la storia naturale della malattia? Il risultato comporta l'adozione di strategie di intervento efficaci? E' utile per i famigliari del paziente? Dà origine a problematiche di tipo etico e/o sociale?

- L'utilità clinica di un determinato marcatore genetico-molecolare deve essere valutata e validata all'interno di specifici scenari clinici

- Punto di partenza di un corretto uso della diagnostica molecolare in citologia tiroidea è conoscere la propria casistica ed i suoi punti deboli

GRAZIE PER L'ATTENZIONE!