Società Medico Chirurgica di Ferrara

dal 1846

APPROCCIO DIAGNOSTICO-TERAPEUTICO AL CARCINOMA DIFFERENZIATO DELLA TIROIDE

Sabato 6 aprile 2013
Aula Magna Nuovo Arcispedale S. Anna

Ruolo dell'analisi genetica

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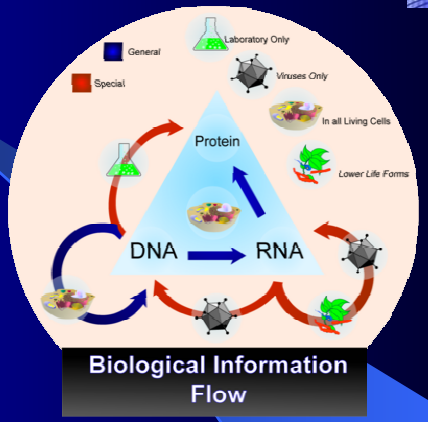
Università degli Studi di Ferrara

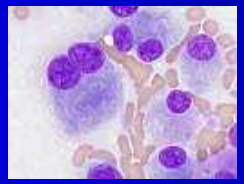
Genetic analysis

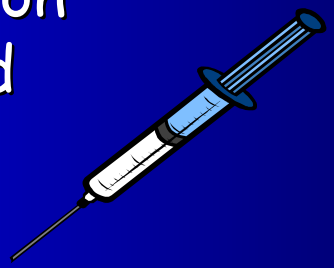
MOLECULAR BIOLOGY
study of biology at molecular level



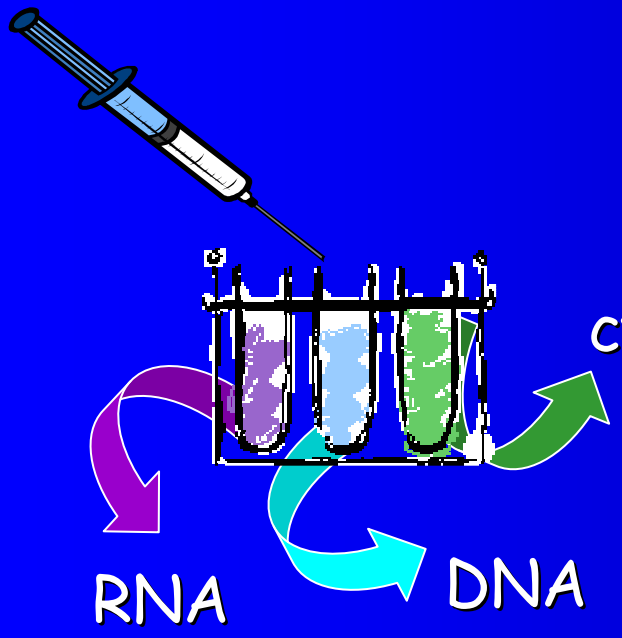
pathology

Does it improve cytological fine needle aspiration diagnosis of thyroid nodules?





Genetic analysis



rearrangement studies

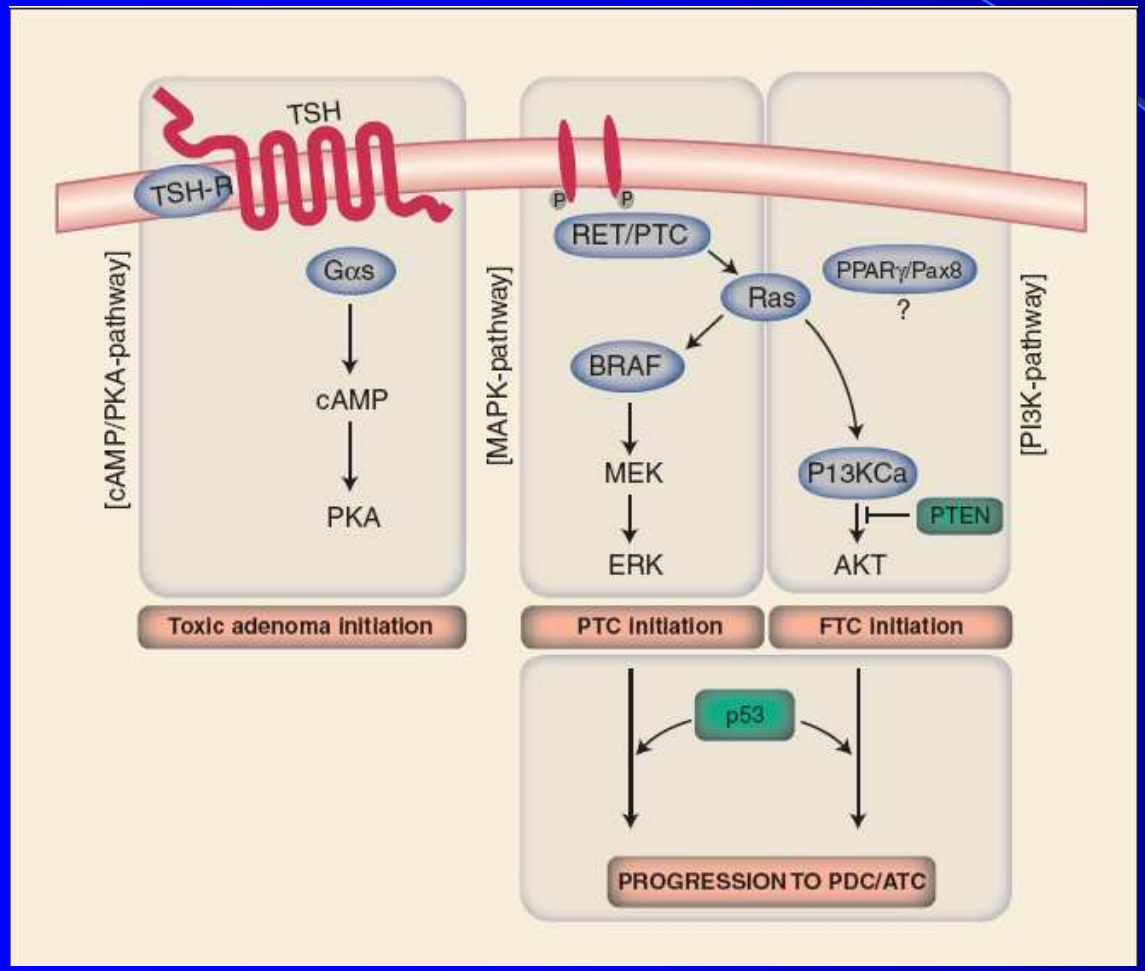
somatic mutation analysis

15-20% FNAB inconclusive or unable to discriminate between follicular adenoma and carcinoma

need for partial or total thyroidectomy for diagnostic purposes

Riesco-Eizaguirre et al. Clin Transl Oncol 2007, 9:686-693

Genetic analysis



Aberrant activation in PTC

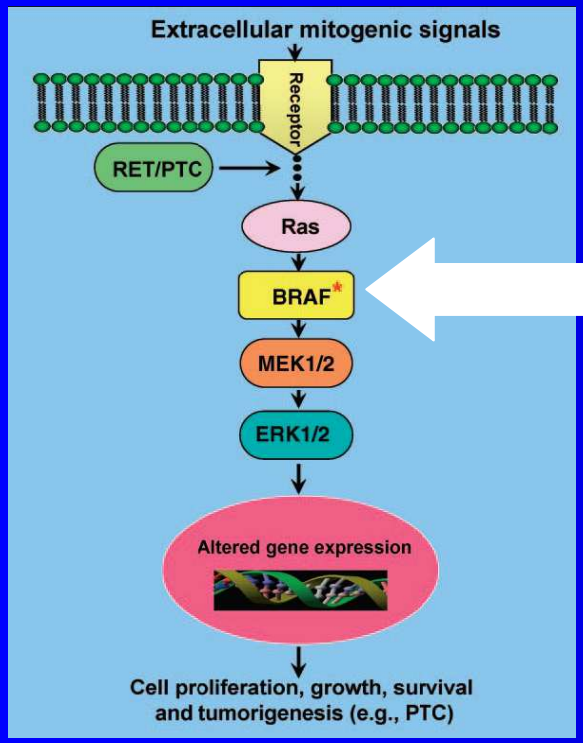
- B-RAF mutations
- RAS mutations
- RET/PTC rearrangements

70%

Riesco-Eizaguirre et al. Clin Transl Oncol 2007, 9:686-693

Genetic analysis

PAPILLARY CARCINOMA



DNA

BRAF V600E point mutation

[K601E and V599Ins]

- ✓ 45-80% of PTC, mainly tall cell and classic hystology
- ✓ ↑ extrathyroidal invasion
- ✓ higher stage
- ✓ ↑ recurrence (with reduced I up-take)

Lupi et al. J Clin Endocrinol Metab. 2007;92:4085

- ✓ ↑ de-differentiation

resticted to PTC

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

Genetic analysis

BRAFV600E molecular test

somatic mutation analysis

pyrosequencing
Kim et al. J Clin Endocrinol Metab, 2011, 96:658

MASA
Pelizzo et al. Clin Chem Lab Med. 2011;49:325

RFLP
Zatelli et al. Eur J Endocrinol 2009, 161:467

direct sequencing
Zatelli et al. Eur J Endocrinol 2009, 161:467

allelic discrimination
Rossi et al. J Clin Endocrinol Metab 2012;97:2354

specific colorimetric mutation detection assay
(Mutector; TrimGen, Sparks, MD)

Xing et al. J Clin Oncol. 2009;27:2977-82

Affordable costs

Dedicated instruments

Experienced personnel

Genetic analysis

BRAFV600E molecular test

469 selected nodules

	Cytology	BRAF	Cytology + BRAF
Sensitivity	77.3	64.0	86.7
Specificity	98.8	100	98.8
PPV	92.1	100	92.9
NPV	95.9	93.7	97.5
Accuracy	95.4	95.4	96.9
K value	0.81±0.02	0.76±0.05	0.88±0.01

Zatelli et al . Eur J Endocrinol 2009, 161:467

EFE 2013

Genetic analysis

BRAFV600E
molecular test

Increased sensitivity for PTC diagnosis in suspected nodules

Zatelli et al . Eur J Endocrinol 2009, 161:467

EFE 2013

Genetic analysis

BRAFV600E molecular test

2421 unselected nodules

	Cytology	BRAF	Cytology + BRAF
Sensitivity	68.5	48.9	96.4
Specificity	96.8	100	96.7
PPV	98.1	100	96.4
NPV	55.2	43.6	96.7

Martina Rossi et al JCEM 2012;97:2354

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Genetic analysis

US findings	Total (2421)	Cancers (233)
Hypoechoic	1173	168
< 1 cm	749	89
> 1 cm	429	79
Isoechoic	1010	38
< 1 cm	341	20
> 1 cm	669	18
Hyperechoic	97	6
< 1 cm	51	1
> 1 cm	46	5
Microcalcifications	675	107
< 1 cm	310	46
> 1 cm	365	61
Blurred margins	62	27
< 1 cm	32	8
> 1 cm	30	19
Intranodular vascularity	84	12
< 1 cm	32	4
> 1 cm	52	8

Clinical findings	Total (2421)	Cancers (233)
Age (<30 or > 60 years)	861	85
< 1 cm	382	32
> 1 cm	479	58
Male gender	514	43
< 1 cm	234	27
> 1 cm	280	16

All clinical/US characteristics suspected for malignancy were investigated

Martina Rossi et al JCEM 2012;97:2354

Genetic analysis

Number of clinical/US findings suspected for malignancy in nodules diagnosed as cancer at histology	
None	4
< 1 cm	3
> 1 cm	1
One	59
< 1 cm	41
> 1 cm	18
Two	79
< 1 cm	62
> 1 cm	17
More than two	91
< 1 cm	34
> 1 cm	57

Even nodules lacking clinical/US findings suspected for malignancy may underlie a thyroid cancer!!!

None of the clinical/US findings suspected for malignancy predicts BRAF status

Martina Rossi et al JCEM 2012;97:2354

Genetic analysis

BRAFV600E
molecular test

Increased sensitivity for PTC diagnosis in unsuspected nodules

Martina Rossi et al JCEM 2012;97:2354

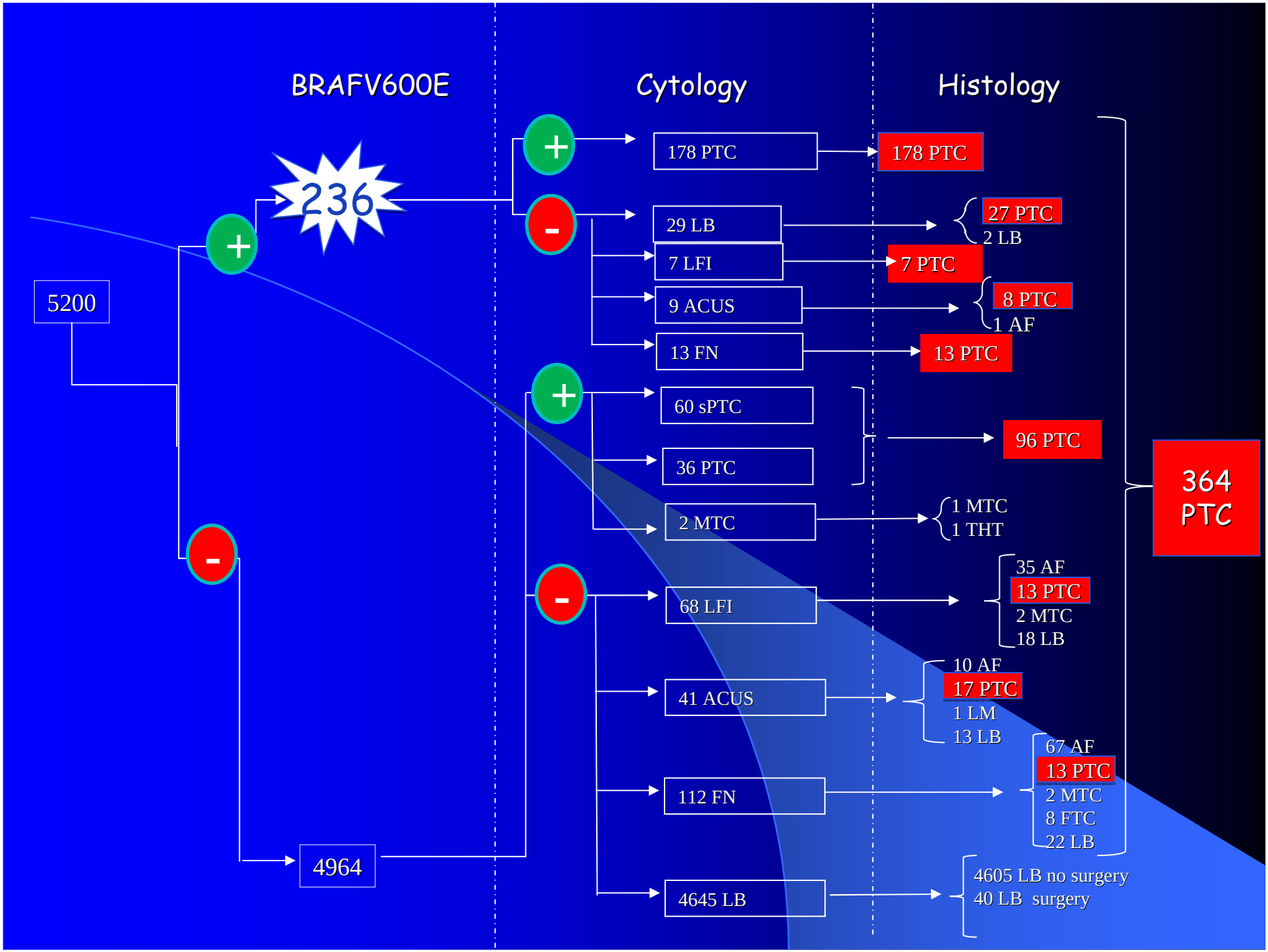
EFE 2013

Genetic analysis

BRAFV600E molecular test

5200 unselected nodules (31/12/2012)

	Cytology	BRAF	Cytology + BRAF
Sensitivity	83,5	70,7	98,8
Specificity	100	99,9	99,9
PPV	100	98,3	98,8
NPV	98,8	98,1	99,9
Accuracy	99	98,1	99,9


BRAFV600E
Cytology
Histology
5200
236
+
-
4964
178 PTC
178 PTC
29 LB
27 PTC
2 LB
7 LFI
7 PTC
9 ACUS
8 PTC
1 AF
13 FN
13 PTC
+
60 sPTC
36 PTC
96 PTC
2 MTC
1 MTC
1 THT
-
68 LFI
35 AF
13 PTC
2 MTC
18 LB
41 ACUS
10 AF
17 PTC
1 LM
13 LB
112 FN
67 AF
13 PTC
2 MTC
8 FTC
22 LB
4645 LB
4605 LB no surgery
40 LB surgery
364 PTC

Genetic analysis

McNEMAR TEST
(p<0,05)

COMBINED METHODS → K = 0,999

BRAF MUTATION ANALYSIS INCREASES SENSITIVITY, NPV and ACCURACY OF CYTOLOGY IN THE DIAGNOSIS OF PTC

Genetic analysis

What about other mutations ?
and other malignant lesions?

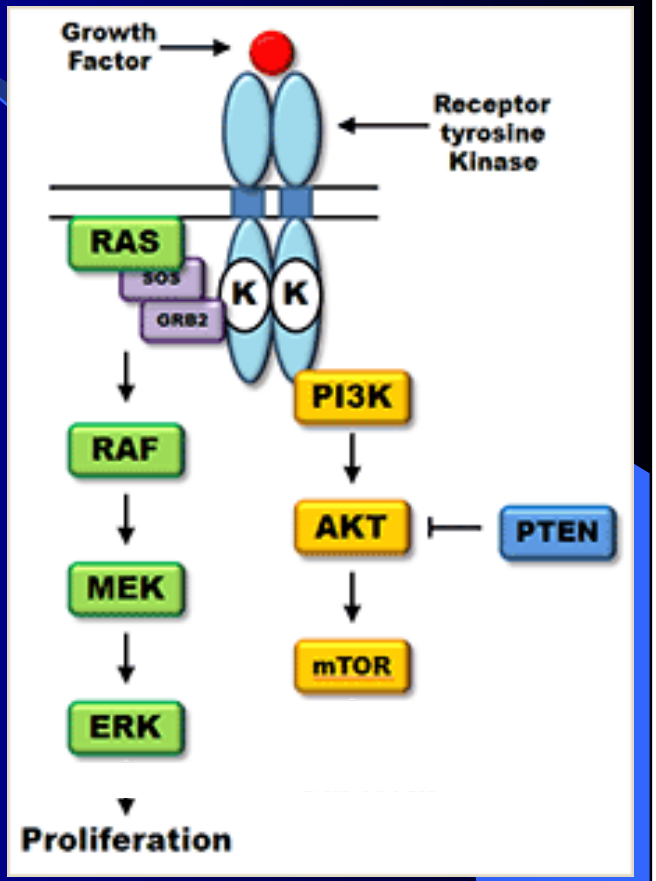
Genetic analysis

H-RAS, N-RAS, K-RAS

Somatic mutations frequently found in human cancers

K-RAS = pancreas, colon , lung
H-RAS = bladder and urinary tract
N-RAS, H-RAS e K-RAS = thyroid tumors

Bos JL Cancer Research 1989;49:4682



Genetic analysis

RAS MUTATIONS IN THYROID NODULES

CODONS 12, 13 and 61
(most frequently N-RAS, codon 61)

⇨ RAS protein is constitutively active

RAS mutations are prevalent in lesions with follicular pattern :

- ✓ follicular carcinomas
- ✓ follicular adenomas
- ✓ follicular variant of papillary carcinoma

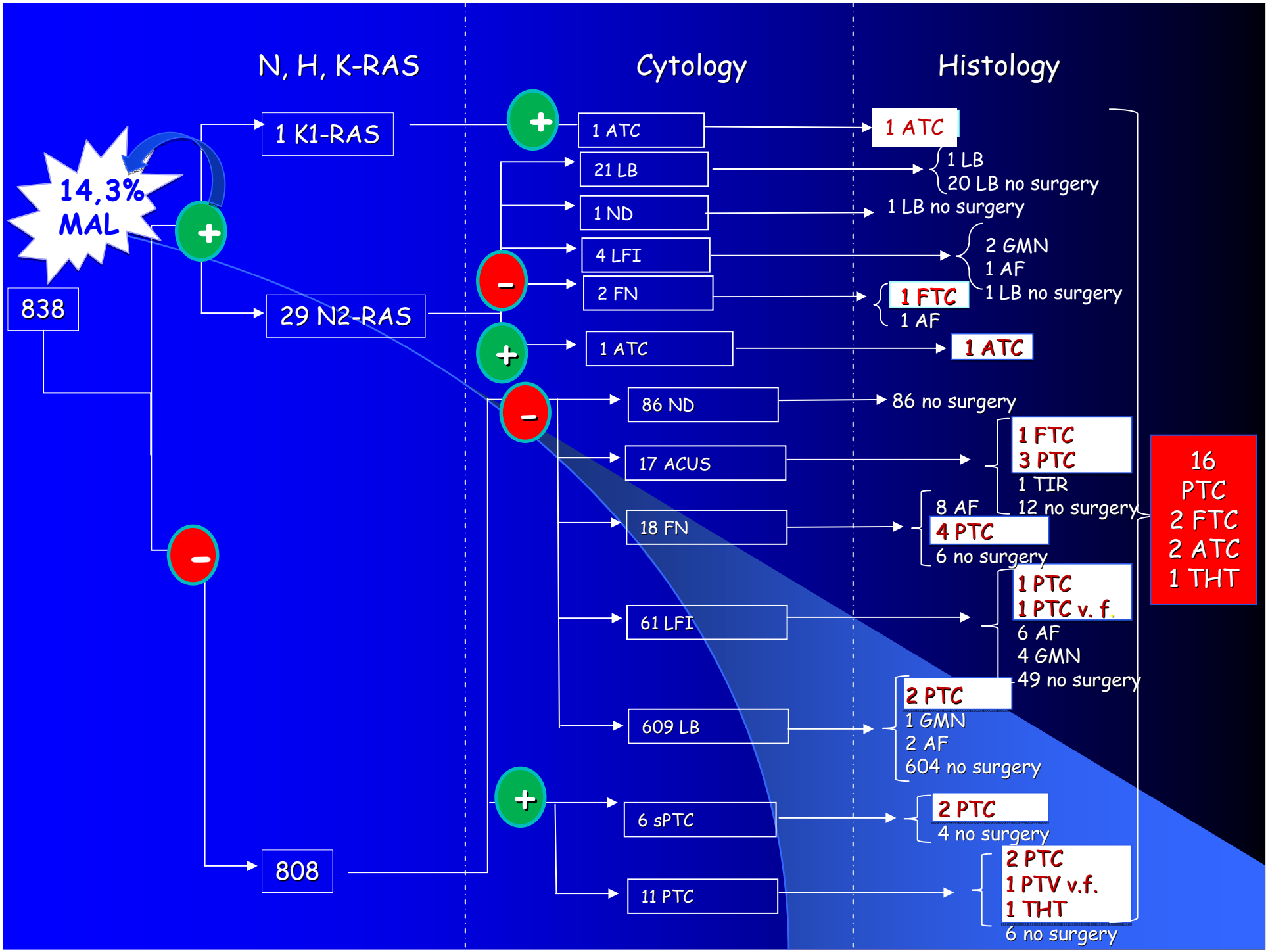
Vasko et al. J Clin Endocrinol Metab 2003, 88:2745

Genetic analysis

RAS molecular test

838 unselected nodules (31/12/2012)

	Cytology	RAS	Cytology + RAS
Sensitivity	46,7	14,3	20
Specificity	99,8	99,4	100
PPV	77,8	37,5	97,3
NPV	99	87,8	98,6
Accuracy	98,8	97,3	98,6


N, H, K-RAS
Cytology
Histology
14,3%
MAL
838
1 K1-RAS
29 N2-RAS
808
1 ATC
21 LB
1 ND
4 LFI
2 FN
1 ATC
86 ND
17 ACUS
18 FN
61 LFI
609 LB
6 sPTC
11 PTC
1 ATC
1 LB
20 LB no surgery
1 LB no surgery
2 GMN
1 AF
1 LB no surgery
1 FTC
1 AF
1 ATC
86 no surgery
1 FTC
3 PTC
1 TIR
12 no surgery
8 AF
4 PTC
6 no surgery
1 PTC
1 PTC v. f.
6 AF
4 GMN
49 no surgery
2 PTC
1 GMN
2 AF
604 no surgery
2 PTC
4 no surgery
2 PTC
1 PTV v.f.
1 THT
6 no surgery
16
PTC
2 FTC
2 ATC
1 THT

Genetic analysis

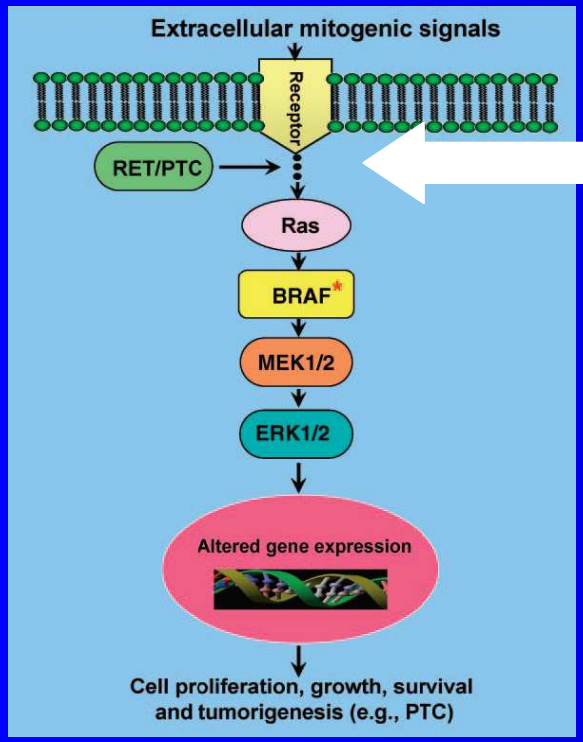
McNEMAR TEST
(p<0,05)

COMBINED METHODS → K = 0,98

RAS MUTATION ANALYSIS ALLOWS TO CORRECTLY IDENTIFY BENIGN NODULES and TO SELECT MALIGNANT LESIONS EVENTUALLY NOT IDENTIFIED BY CYTOLOGY

Genetic analysis

PAPILLARY CARCINOMA



RET/PTC1 and RET/PTC3 paracentric inversions

- ✓ 20% of PTC, mainly classical histology
- ✓ younger age
- ✓ radiation exposure
- ✓ ↑ lymph node metastases
- ✓ lower stage (micro)
- ✓ found in adenomas and benign lesions

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

Genetic analysis

RET/PTC rearrangement

transforms thyroid cells in culture

Santoro et al. Cell Growth Differ 1993;4:77–84

gives rise to thyroid carcinomas in transgenic mice

Jhiang et al. Endocrinology 1996;137:375–378
Santoro et al. Oncogene 1996;12:1821–1826
Powell et al. Cancer Res 1998;58:5523–5528

requires a functional BRAF signaling

Melillo et al. J Clin Invest 2005;115:1068–1081

inhibits thyroid-specific gene expression

increases cell proliferation

Mitsutake et al. Cancer Res 2005;65: 2465–2473

tumorigenesis

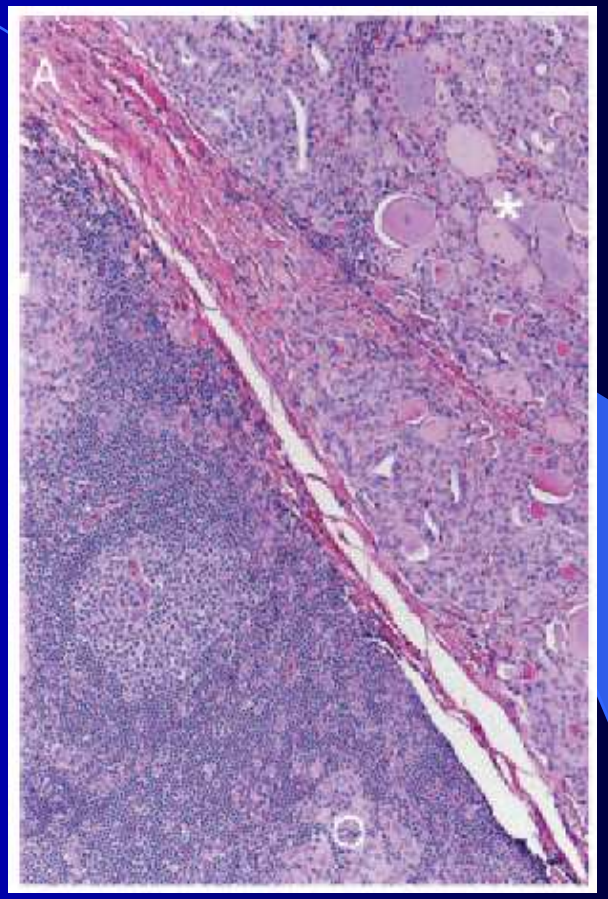
Genetic analysis

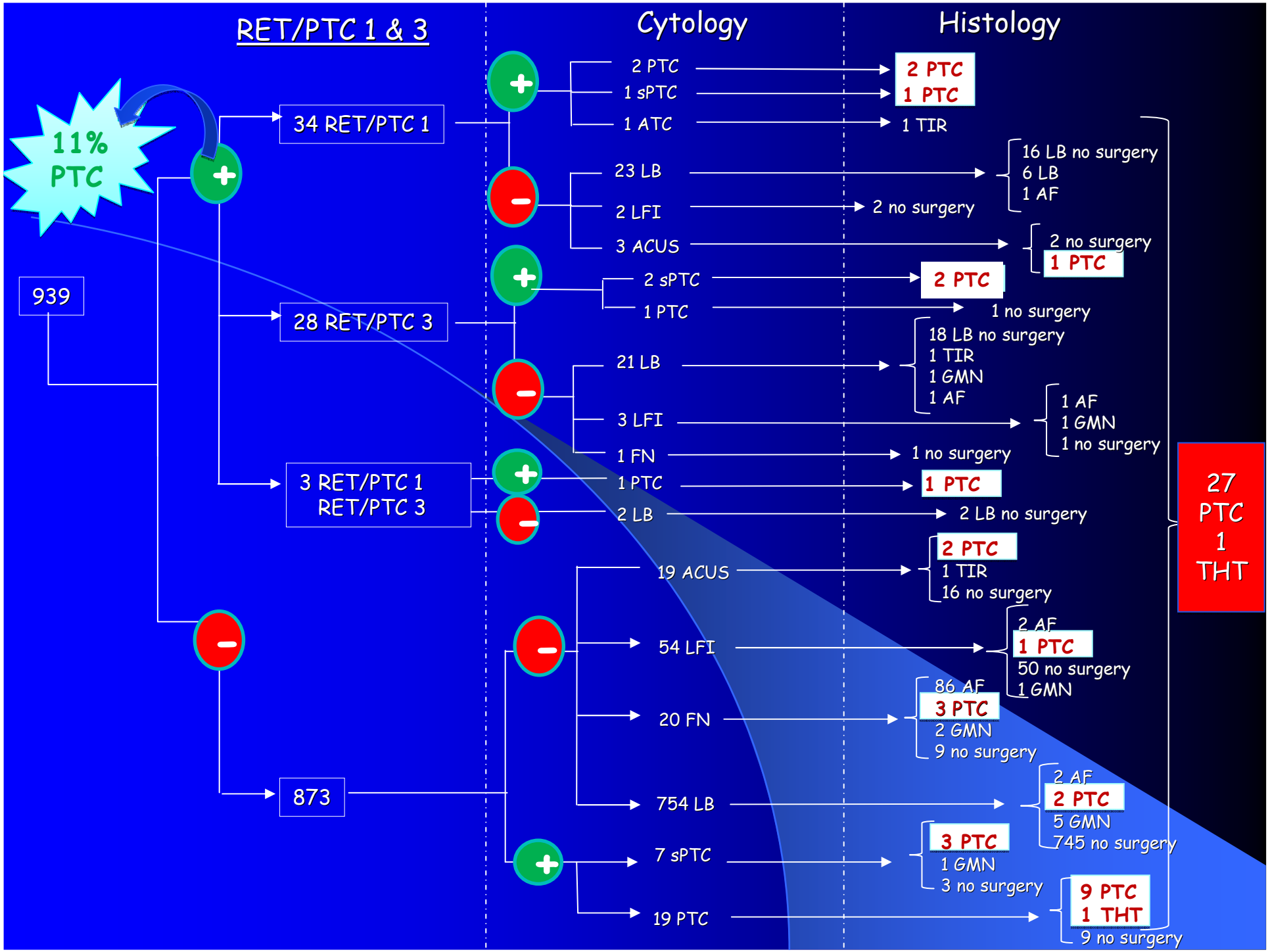
RET/PTC rearrangement

Found in 62% of HT

Sheils OM et al. Int J Surg Pathol 2000 ,8:185–189
Wirtschafter A et al. Laryngoscope 1997, 107:95–100
Rhoden KJ et al. J Clin Endocrinol Metab 2006, 91: 2414–2423

occult neoplasm ?




RET/PTC 1 & 3
Cytology
Histology
11% PTC
939
34 RET/PTC 1
28 RET/PTC 3
3 RET/PTC 1
RET/PTC 3
873
2 PTC
1 sPTC
1 ATC
2 PTC
1 PTC
1 TIR
23 LB
2 LFI
3 ACUS
16 LB no surgery
6 LB
1 AF
2 no surgery
2 no surgery
1 PTC
2 sPTC
1 PTC
2 PTC
1 no surgery
21 LB
3 LFI
1 FN
18 LB no surgery
1 TIR
1 GMN
1 AF
1 AF
1 GMN
1 no surgery
1 no surgery
1 PTC
2 LB
1 PTC
2 LB no surgery
19 ACUS
54 LFI
20 FN
754 LB
7 sPTC
19 PTC
2 PTC
1 TIR
16 no surgery
2 AF
1 PTC
50 no surgery
1 GMN
86 AF
3 PTC
2 GMN
9 no surgery
2 AF
2 PTC
5 GMN
745 no surgery
3 PTC
1 GMN
3 no surgery
9 PTC
1 THT
9 no surgery
27 PTC 1 THT

Genetic analysis

RET/PTC rearrangements molecular test

939 unselected nodules (31/12/2012)

	Cytology	RET/PTC	Cytology + RET/PTC
Sensitivity	69	22,2	42,9
Specificity	99,8	98,4	99,9
PPV	90,9	31,6	85,7
NPV	99	97,7	99,1
Accuracy	98,8	96,4	99

Genetic analysis

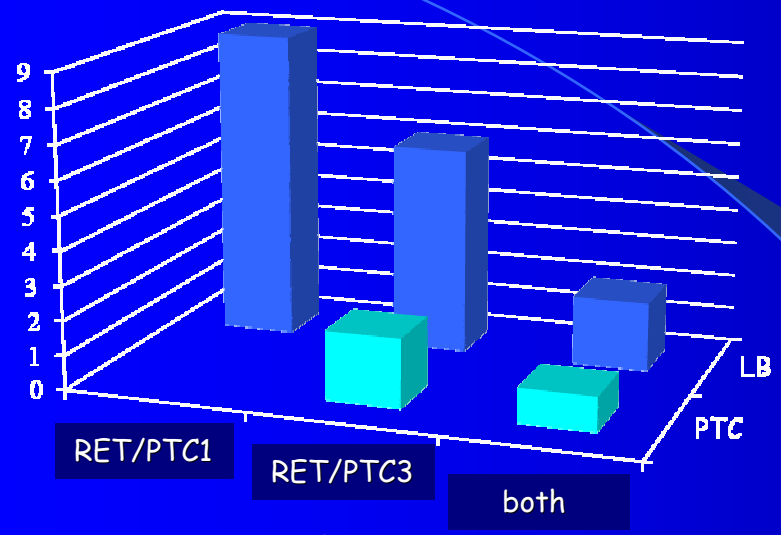
McNEMAR TEST
(p<0,05)

COMBINED METHODS → K = 0,99

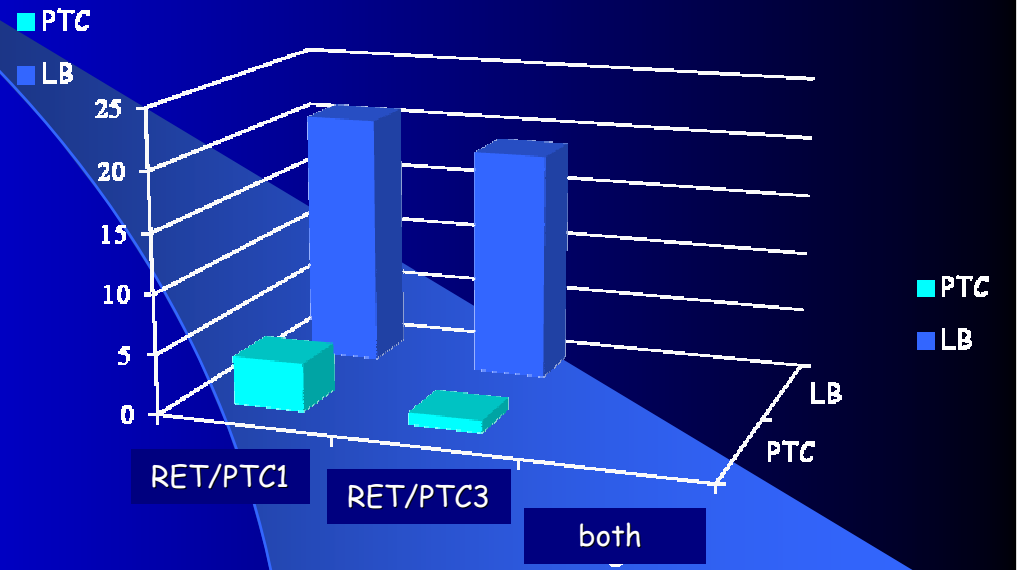
RET/PTC REARRANGEMENTS INVESTIGATION ALLOWS TO CORRECTLY IDENTIFY BENIGN LESIONS

Genetic analysis

RET/PTC REARRANGEMENTS & THYROIDITIS



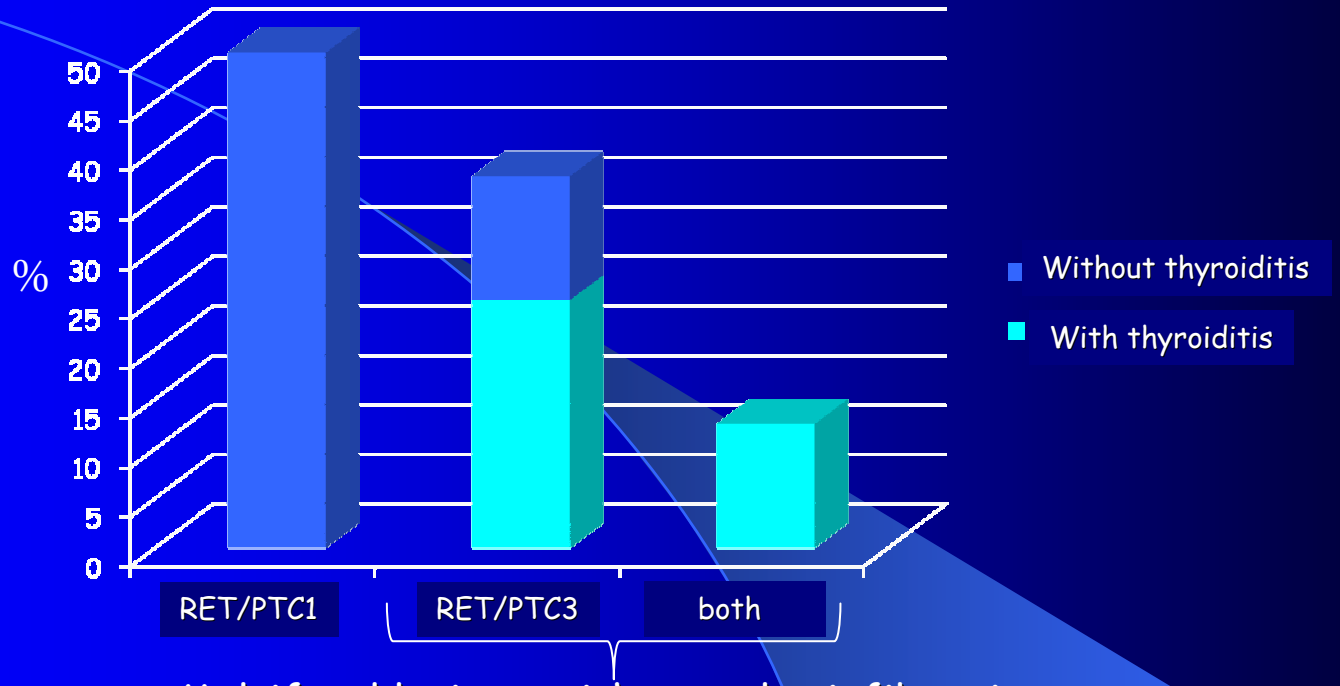
with thyroiditis



without thyroiditis

Genetic analysis

RET/PTC REARRANGEMENTS & THYROIDITIS

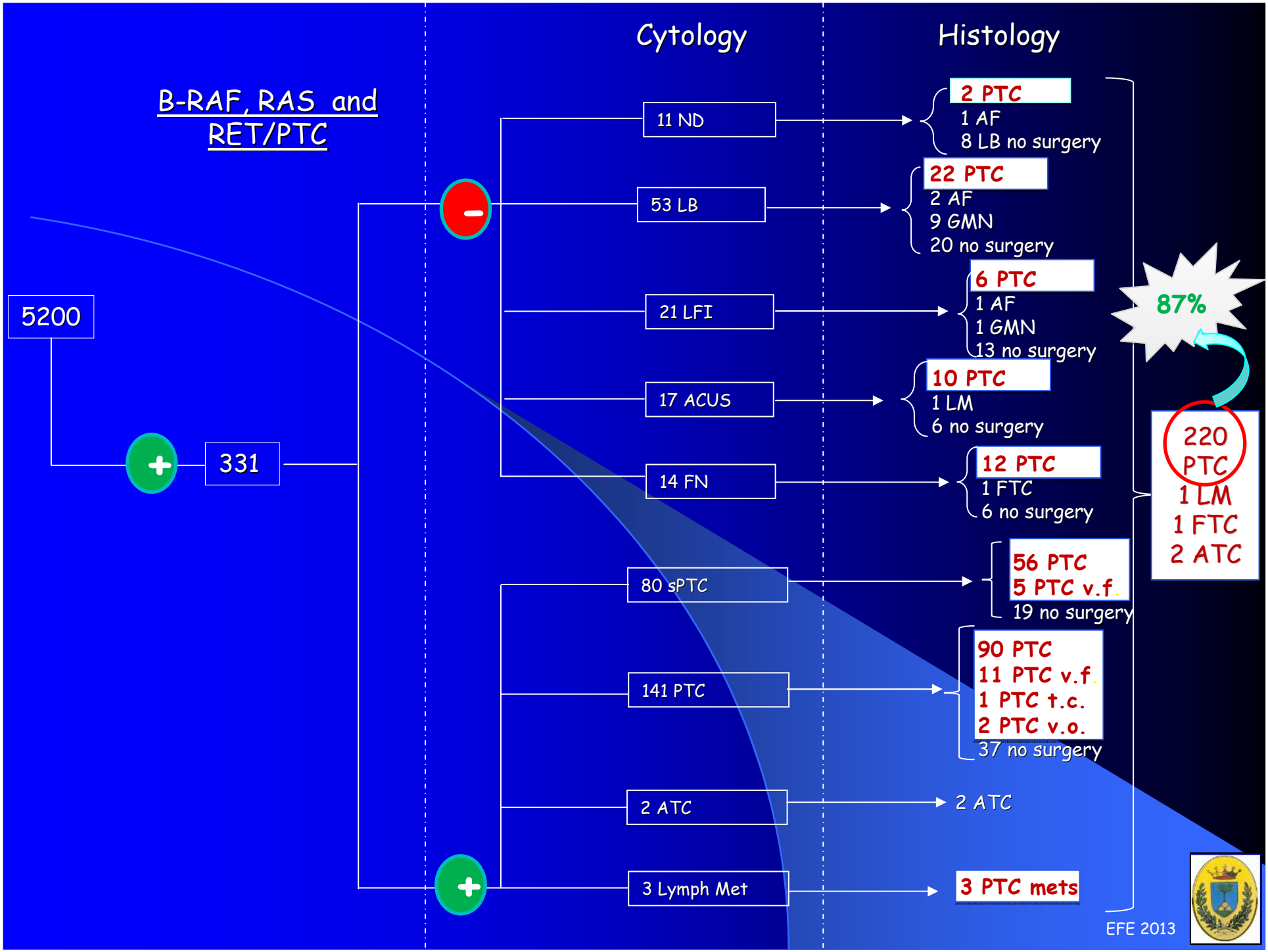


Multifocal lesions, with capsular infiltration,
frequently associated with lymphnode metastases

RET/PTC rearrangements identify more aggressive cancers when associated with chronic thyroiditis

Genetic analysis

What about combined molecular analysis ?


B-RAF, RAS and RET/PTC
Cytology
Histology
5200
331
11 ND
53 LB
21 LFI
17 ACUS
14 FN
80 sPTC
141 PTC
2 ATC
3 Lymph Met
2 PTC
1 AF
8 LB no surgery
22 PTC
2 AF
9 GMN
20 no surgery
6 PTC
1 AF
1 GMN
13 no surgery
10 PTC
1 LM
6 no surgery
12 PTC
1 FTC
6 no surgery
56 PTC
5 PTC v.f.
19 no surgery
90 PTC
11 PTC v.f.
1 PTC t.c.
2 PTC v.o.
37 no surgery
2 ATC
3 PTC mets
87%
220 PTC
1 LM
1 FTC
2 ATC
EFE 2013

Genetic analysis

ANY
molecular test

	Cytology	Genetic analysis	Cytology + genetic analysis
Sensitivity	62,3	52,3	59,4
Specificity	99,6	99,7	100
PPV	94,3	94,4	100
NPV	96,5	95,7	97,7
Accuracy	96,4	96,4	97,8

Genetic analysis

McNEMAR TEST
(p<0,05)

COMBINED METHODS → K = 0,97

COMBINED INVESTIGATION OF B-RAF and RAS MUTATIONS AND OF RET/PTC REARRANGEMENTS ALLOWS TO CORRECTLY IDENTIFY A GREATER NUMBER OF THYROID CANCERS

Genetic analysis

5200

658 indeterminate lesions
12,7%

230 FN
34,9%

131 surgery
56,9%

428 ACUS
65,1%

130 surgery
30,3%

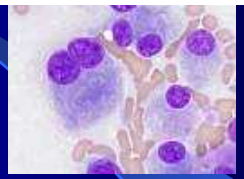
HISTOLOGY % (n=261)	FN (27)	GENETIC ANALYSIS + (13)	ACUS (46)	GENETIC ANALYSIS + (16)
PTC	20,6	48,1	35,3	34,8
FTC	6,1	4,7	0,0	
MTC	1,5		1,5	
Other k	0,0		0,7	
AF	53,4		34,6	
LB	17,6		27,7	

Genetic analysis allows the diagnosis of malignancy in indeterminate lesions

Genetic analysis

THEREFORE

FNAB material



molecular tests

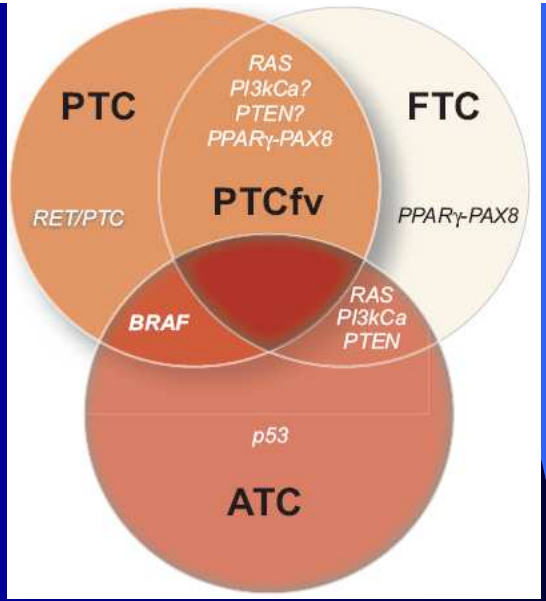
- ✓ increase diagnostic sensitivity of cytology for PTC
- ✓ helps in identifying benign lesions
- ✓ influences surgical approach
- ✓ allows detection of minimal disease metastatic to cervical lymph nodes

Genetic analysis

	BRAF	RET/PTC	RAS	PI3KCa*	PTEN	PPARγ/PAX8
PTC	29–83%	2.5–59%**	–	–	–	–
PTCfv	–	–	5–15%	15%	2%	37.5%
FTC	–	–	7–62%	8–42%	6–7%	36–45%
FA	–	14%	9–11%	8–23%	–	4–33%
ATC/PDC	10–35%	–	50–55%	54%	16%	–
Extrathyroid extension	Yes	No	–	–	–	–
Increased recurrence risk	Yes	No	–	–	–	–
Poor survival	?	No	Yes	–	–	–

Riesco-Eizaguirre et al. Clin Transl Oncol 2007; 9:686

molecular biology can help



Genetic analysis

BUT

Molecular testing is not sufficient to detect all malignant cases

30% PTC
20% FTC
>50% oncocytic FTC

→ no known mutations!!!

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

THANKS

Section of Endocrinology
Dept. of Medical Sciences
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Ettore degli Uberti