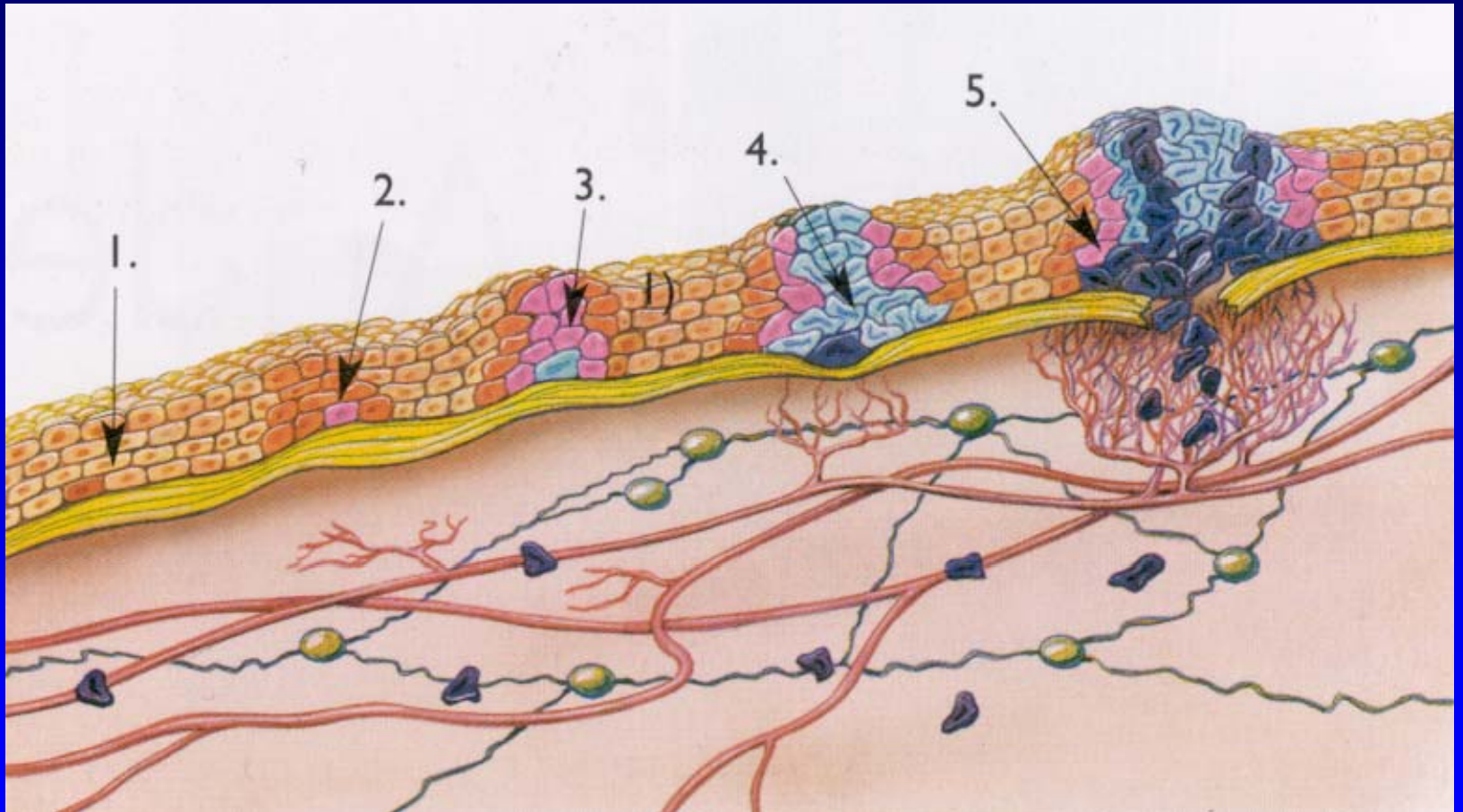
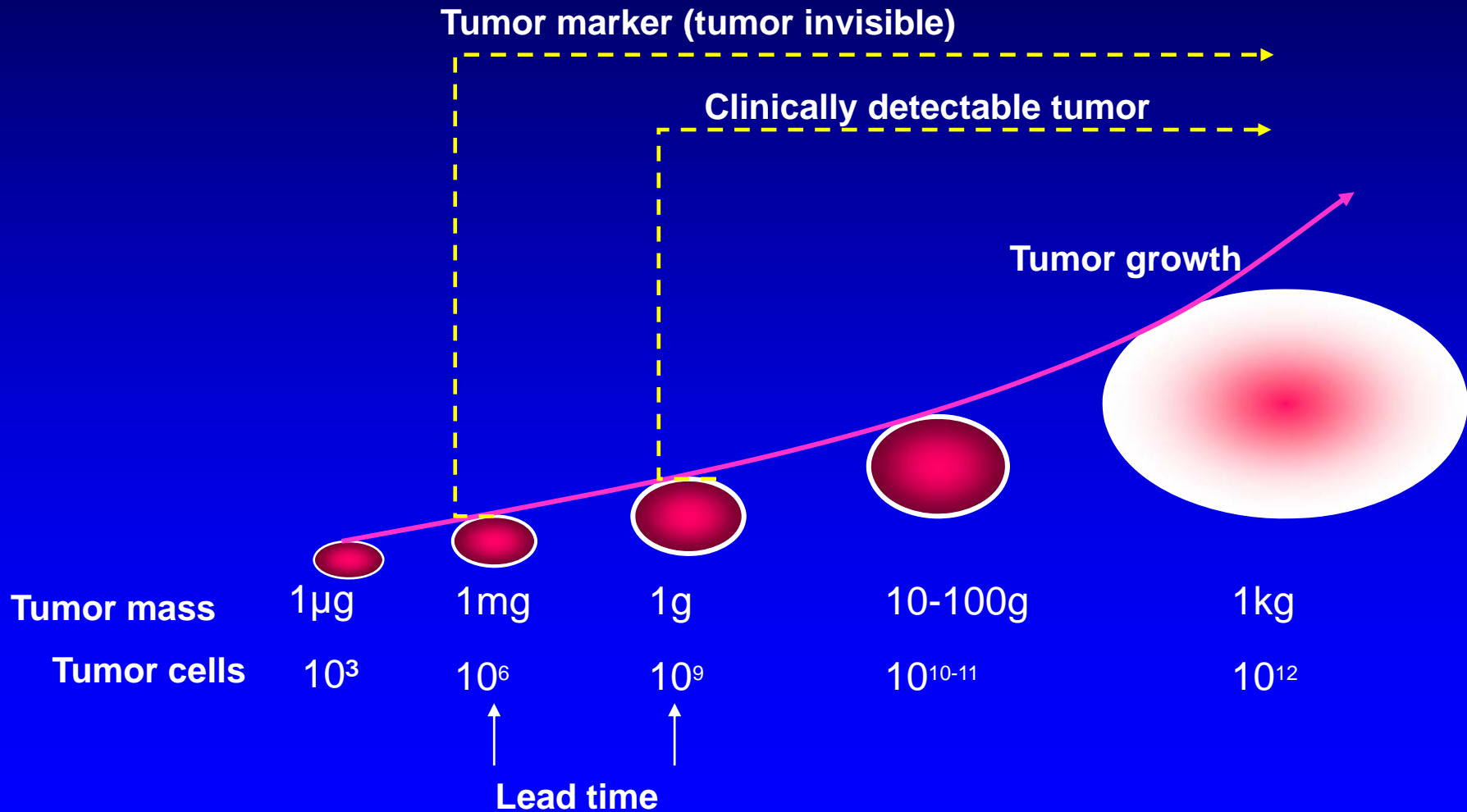


# **Evidence for the clinical use of tumor markers in clinical practice**

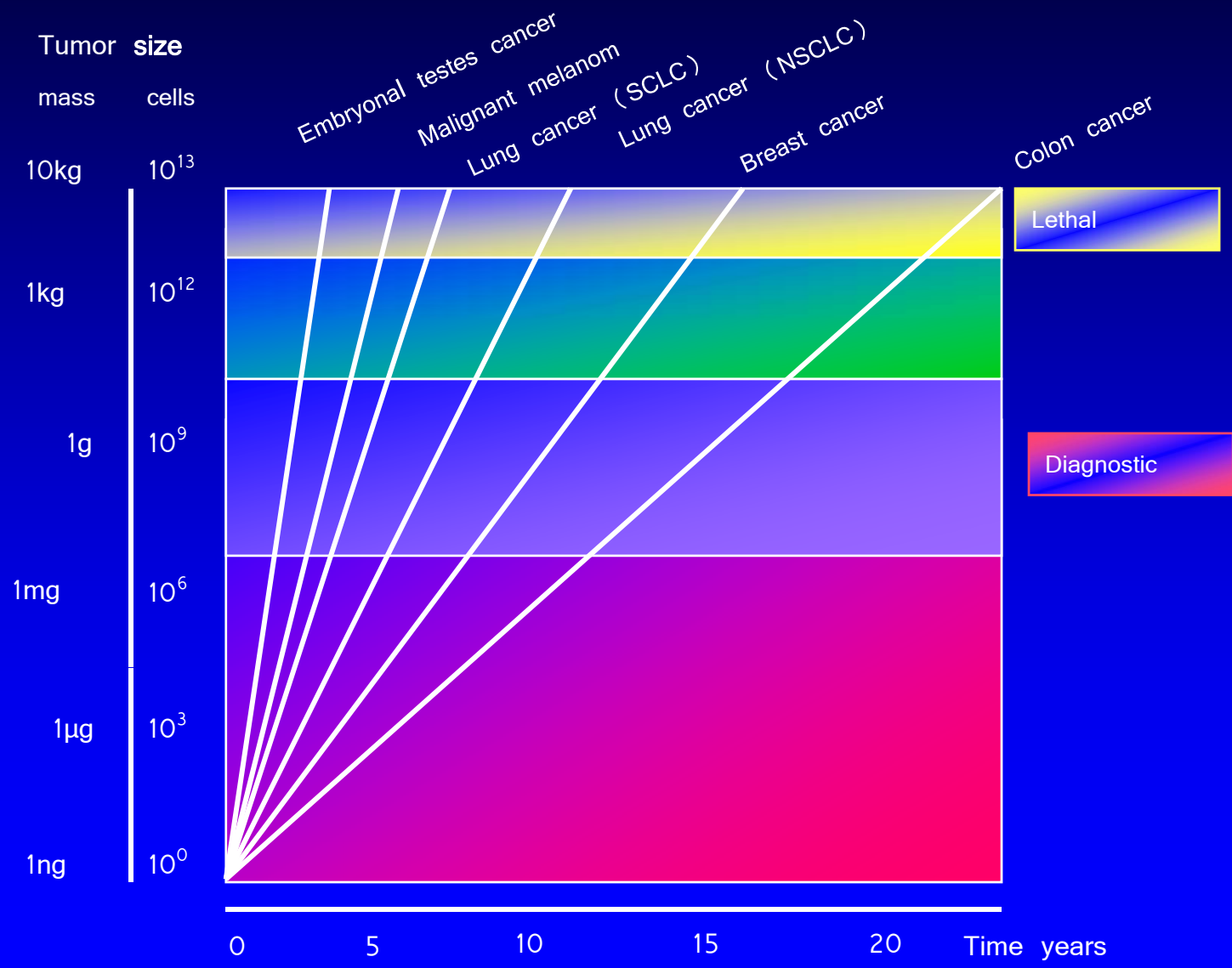
Roland Einarsson



# Measurement of the Growth of Malignant Tumors



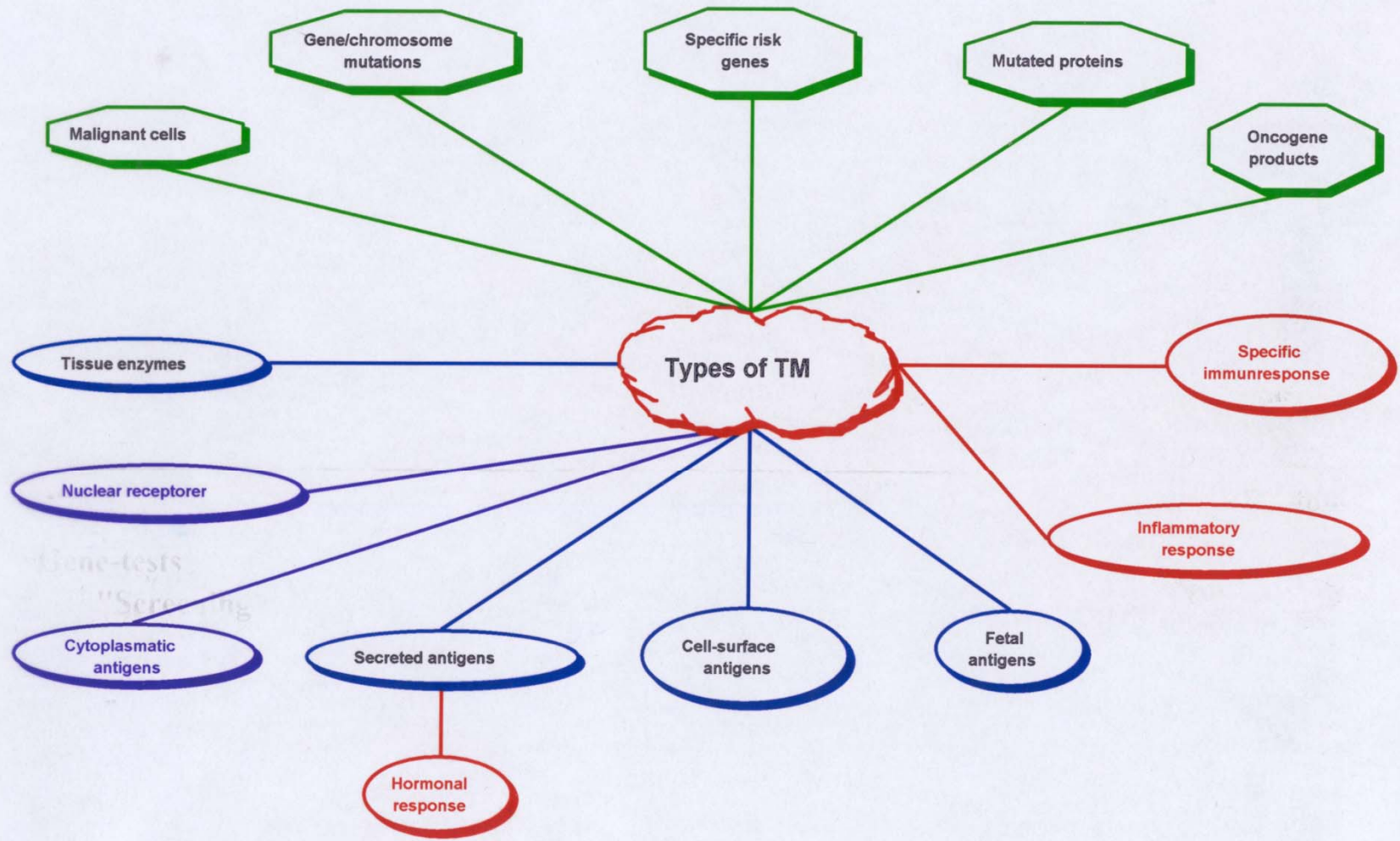
# Tumor Growth



# **5 year survival in the most common carcinomas**

<b>Lung cancer</b>	<b>5-15 (%)</b>
<b>Colorectal cancer</b>	<b>60-65 (%)</b>
<b>Breast cancer</b>	<b>85-90 (%)</b>
<b>Prostate cancer</b>	<b>&gt;90 (%)</b>

# What is a tumor marker?



# Clinically used serum-based tumor markers

Cancer Site	Diagnostic Markers
Breast cancer	CA15-3, CEA, Her-2, TPS/TPA
Ovarian cancer	CA125, CA72-4, TPS
Cervical cancer	SCC, CEA, HR HPV (cervical swab)
Colorectal cancer	CEA, CA 242, TPA
Pancreatic cancer	CA19-9, CA242
Head and neck cancer	CEA, SCC, HPV (Oral Mucosal swab)
Prostate cancer	PSA, fPSA, ratio fPSA/PSA
Lung cancer	
SCLC	NSE, ProGRP
NSCLC	CYFRA, CEA, SCC

# Current clinical/biomarker tests in regular use for screening/case findings in cancer

PSA – prostate cancer

CA125 – ovarian cancer

NMP22 – bladder cancer

Mammography – breast cancer

PAP smear/ HPV HR– cervical cancer

# The ideal tumor marker should detect

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- the presence of a tumor
- its malignant potential
- its stage in the progression pathway
- the extent of metastatic spread

# Tumor marker limitations

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- Lack of specificity
- Lack of sensitivity for early malignancy
- Markers are rarely elevated in all cancers of a particular type
- No marker has absolute organ specificity

# Practice guidelines and recommendations for use of tumor markers in the clinic

Internal Quality Control (IQC)

External Quality Assessment (EQA)

## Expert Organizations Preparing Guidelines

National Academy of Clinical Biochemistry (NACB)

European Group on Tumor Markers (EGTM)

American Society for Clinical Oncology (ASCO)

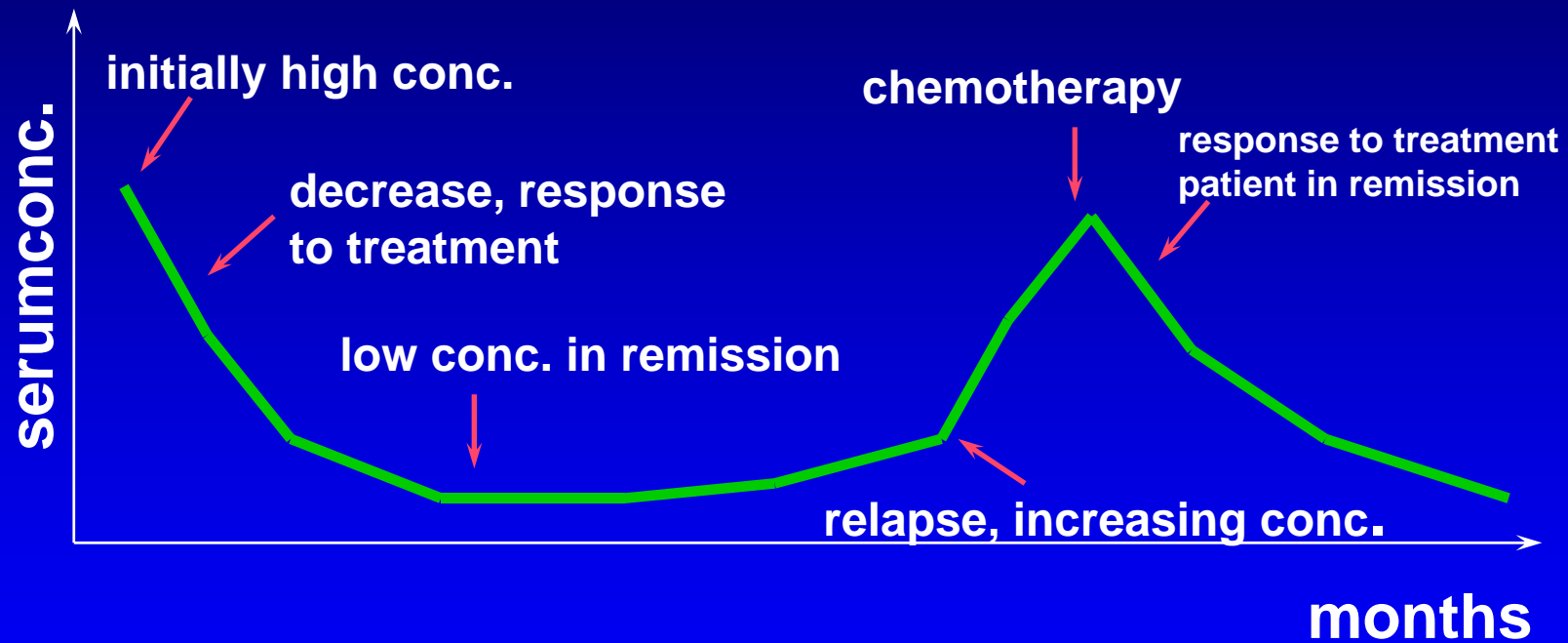
American Cancer Society (ACS)

Clinical Laboratory Improvement Act (CLIA)

# Uses and potential uses of cancer biomarkers

- 1. Screening/Case findings**
- 2. Aiding diagnosis**
- 3. Assessing prognosis**
- 4. Predicting treatment response and recurrence**
- 5. Monitoring patients with diagnosed disease**

# Behaviour of a tumor marker during patient follow-up



# Equations applied in oncological diagnostics

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{true positives} + \text{false negatives}} \times 100$$

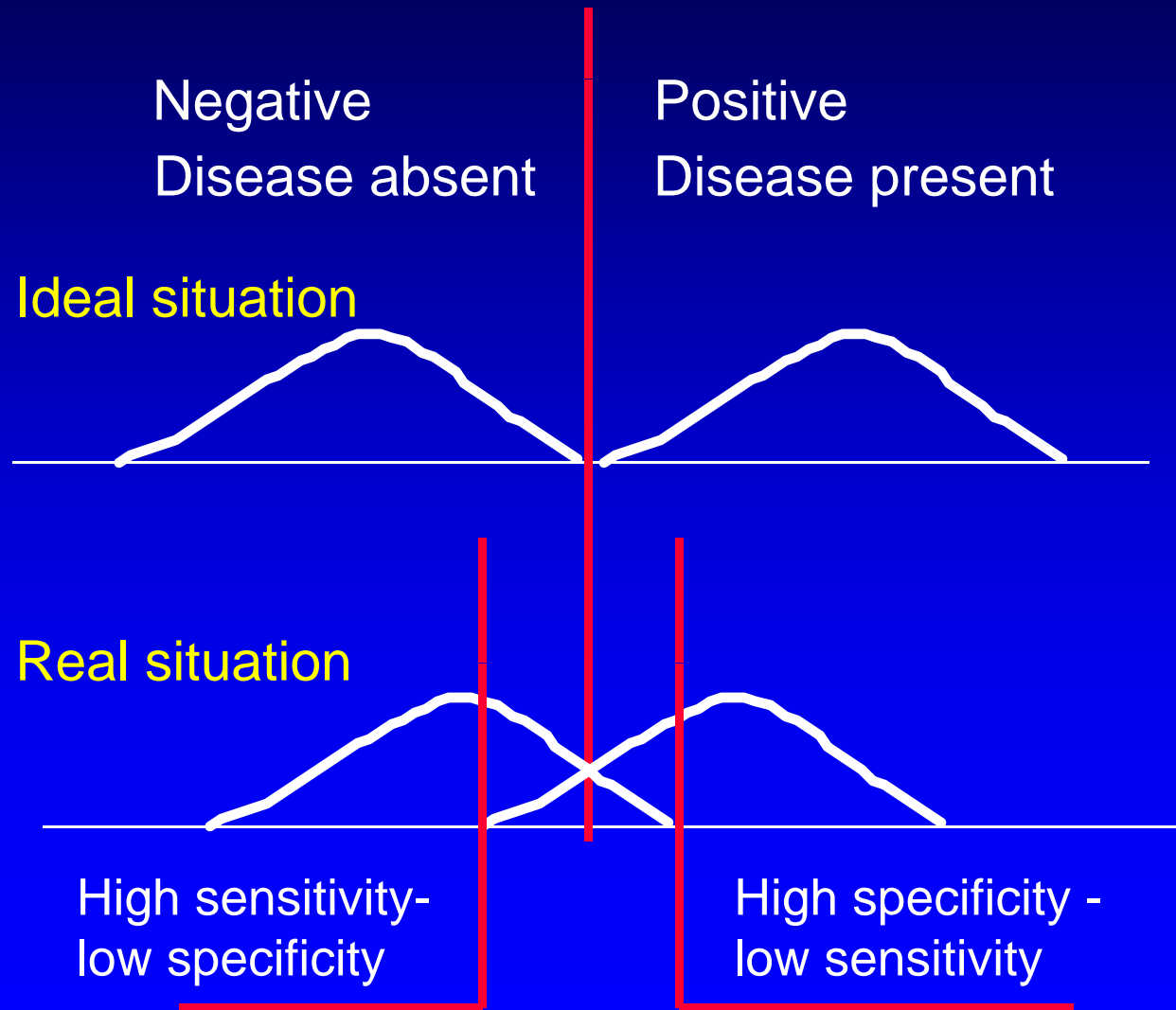
$$\text{Specificity} = \frac{\text{True negatives}}{\text{false positives} + \text{true negatives}} \times 100$$

$$\text{PPV} = \frac{\text{True positives}}{\text{true positives} + \text{false positives}} \times 100$$

$$\text{NPV} = \frac{\text{True negatives}}{\text{true negatives} + \text{false negatives}} \times 100$$

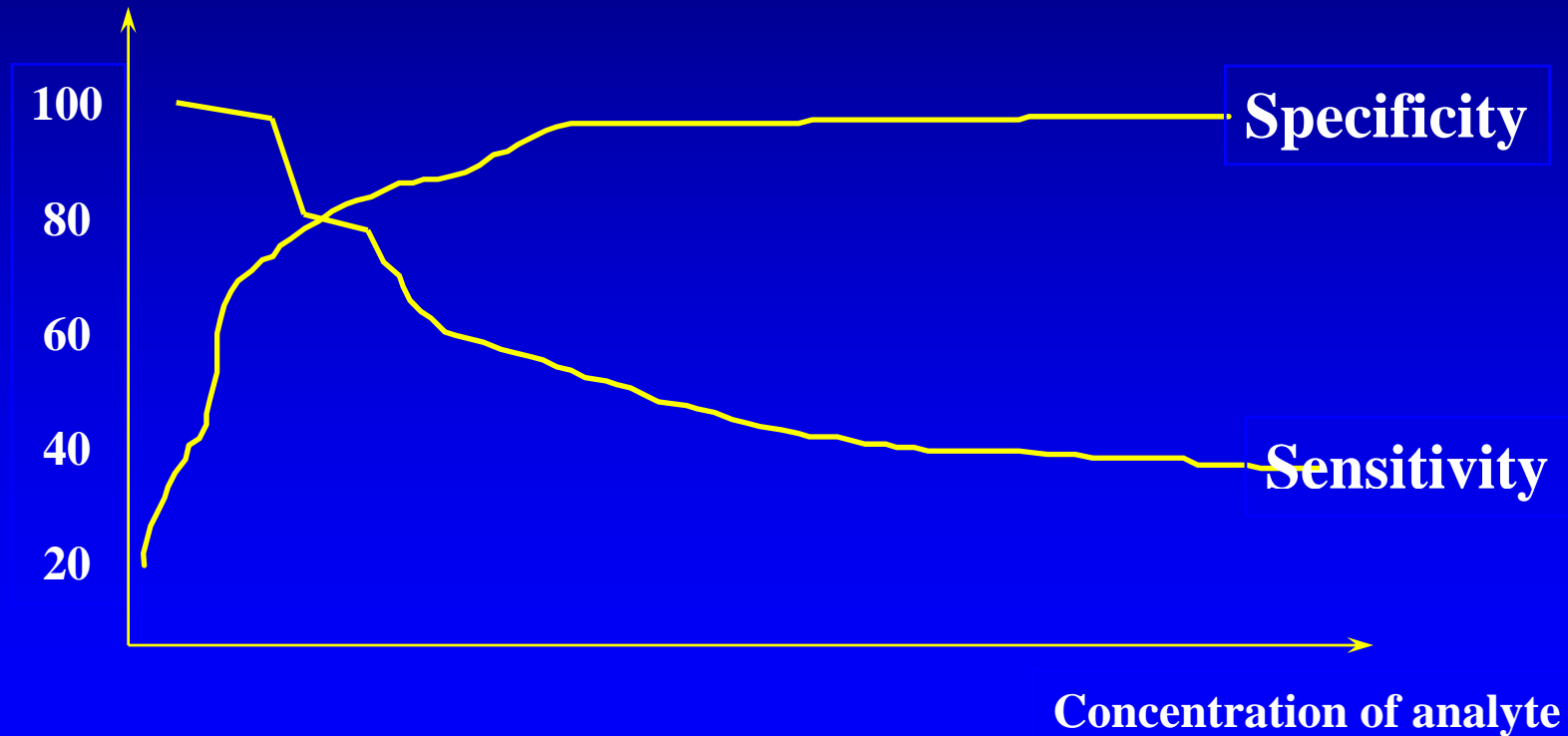
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# Cut-off - Upper reference level



# Sensitivity/Specificity Diagram

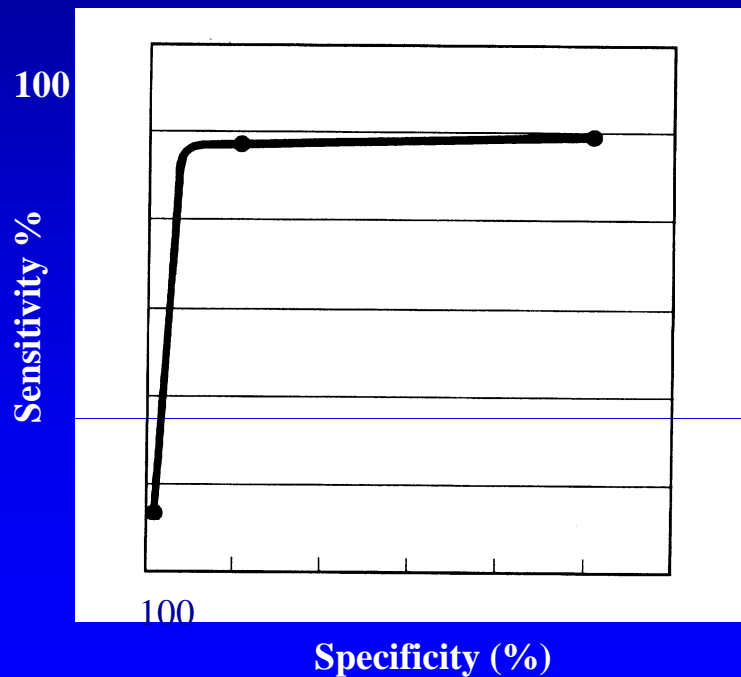
Sensitivity/  
Specificity (%)



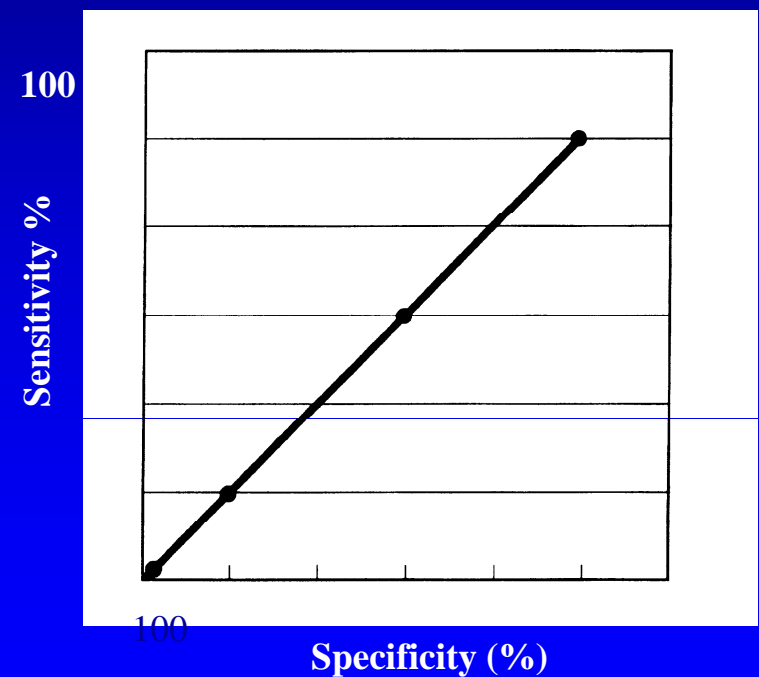
# ROC-curve

## Receiver-Operating Characteristics Sensitivity/specificity diagram

Clinically ideal



Of no clinical value



# Malignant diseases

## Diagnosis:

Subjective signs

Clinical signs

Cytology

Imaging methods

Pathology

Biochemical markers

## Effect of treatment:

Subjective signs

Clinical signs

Imaging methods

Biochemical markers

## Effect of palliative therapy:

Subjective signs

Clinical signs

Imaging methods (only to direct local therapy)

Biochemical markers

# Levels of evidence for grading clinical utility of tumor markers

- “ **Level I** ” – single, high-powered, prospective, randomized and controlled trial or meta-analysis
- “ **Level II** ” – evidence from marker data in relation to prospective therapeutic trial to test therapeutic hypothesis
- “ **Level III** ” – evidence from large prospective/retrospective studies
- “ **Level IV** ” – evidence from small retrospective studies
- “ **Level V** ” – evidence from small pilot studies



## **Tissue-Based Markers**

ER/PR

HER-2/neu

uPA/PAI-1

## **Serum-Based markers**

CA15-3/BR27.29

CEA

TPA/TPS

## **Genetic Markers**

BRCA1/BRCA2

# Tissue Biomarkers in Breast Cancer

- ER, PR analysed in all new Breast Cancer patients for selection of patients to start endocrine therapy (LOE I)
- Her-2 analysed in all new Breast Cancer patients to get information regarding Herceptin treatment in the metastatic setting
- uPA and PAI-1 are independent prognostic factors (nodal metastases, hormone receptor status) (LOE 1)
- Other tissue markers in Breast Cancer are not sufficient evaluated (LOE III-V)

# How to identify high risk node negative breast cancer

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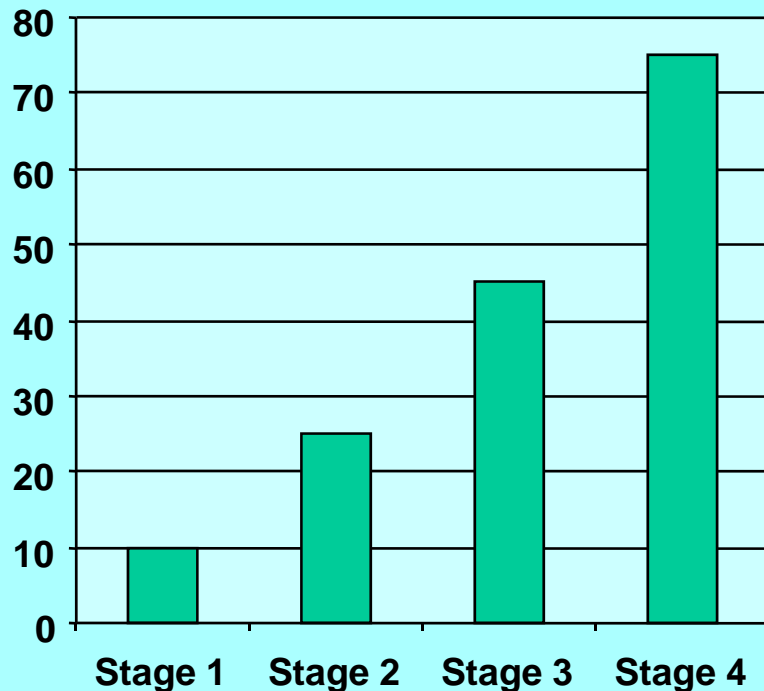
- Node negative comprises 70% of all primary breast cancer patients
- 65–70% remain alive and free of recurrence after 10 years (surgery and radiotherapy)
- 30–35% will recur and die of breast cancer – these patients should be identified and given adjuvant treatment

uPA and PAI-1 might be the answer?

## CA 15-3 in Breast Cancer

- Pre-clinically elevated CA15-3 detects distant metastases in 70% of asymptomatic patients (LOE III)
- Increasing CA15-3 levels might initiate early therapy and positive outcome
- Assessing prognosis — high pre-op levels predict adverse outcome (LOE III)
- CA15-3 should not be used separate for monitoring therapy (combination with CEA, TPA/TPS)

# MUC-1 in different stages of Breast Cancer



- MUC-1 (CA15-3) levels depend on the stage of disease
- The low sensitivity in early stages diminishes the value of MUC-1 in asymptomatic patients
- MUC-1 useful for detection of recurrent disease, but not for primary diagnosis

# Evaluation of Response in Breast Cancer Patients

## UICC

some patients unassessible

experienced clinicians

assessments (variations)

structural damage

expensive

## Tumor Markers

all patients available

objective results

reproducible

early indication of PD

cheap

## CA 15-3 in Breast Cancer

### Limitations

Low incidence of CA 15-3 in early stage disease and CA 15-3 is not sensitive enough to detect micrometastatic disease

25-30% of Breast cancer patients with clinically documented recurrence do not have elevated CA 15-3

# NACB and EGTM recommendations for use of tumor markers in breast cancer

1. The best-validated markers in breast cancer are tissue based – ER and PR for identification of hormone positive patients
2. CA15-3/BR27.29 determinations are useful for the early detection of breast cancer recurrences
3. Decreasing concentrations of CA15-3 are indicative of successful therapeutic response
4. CEA is recommended for the early diagnosis of distant metastases

# Biomarkers in colorectal cancer

## Serum markers

CEA

CA242/CA19-9

Cytokeratins (TPA, TPS)

## Tissue-based markers

Tymidylate synthetase (TS)

uPA/PAI-1

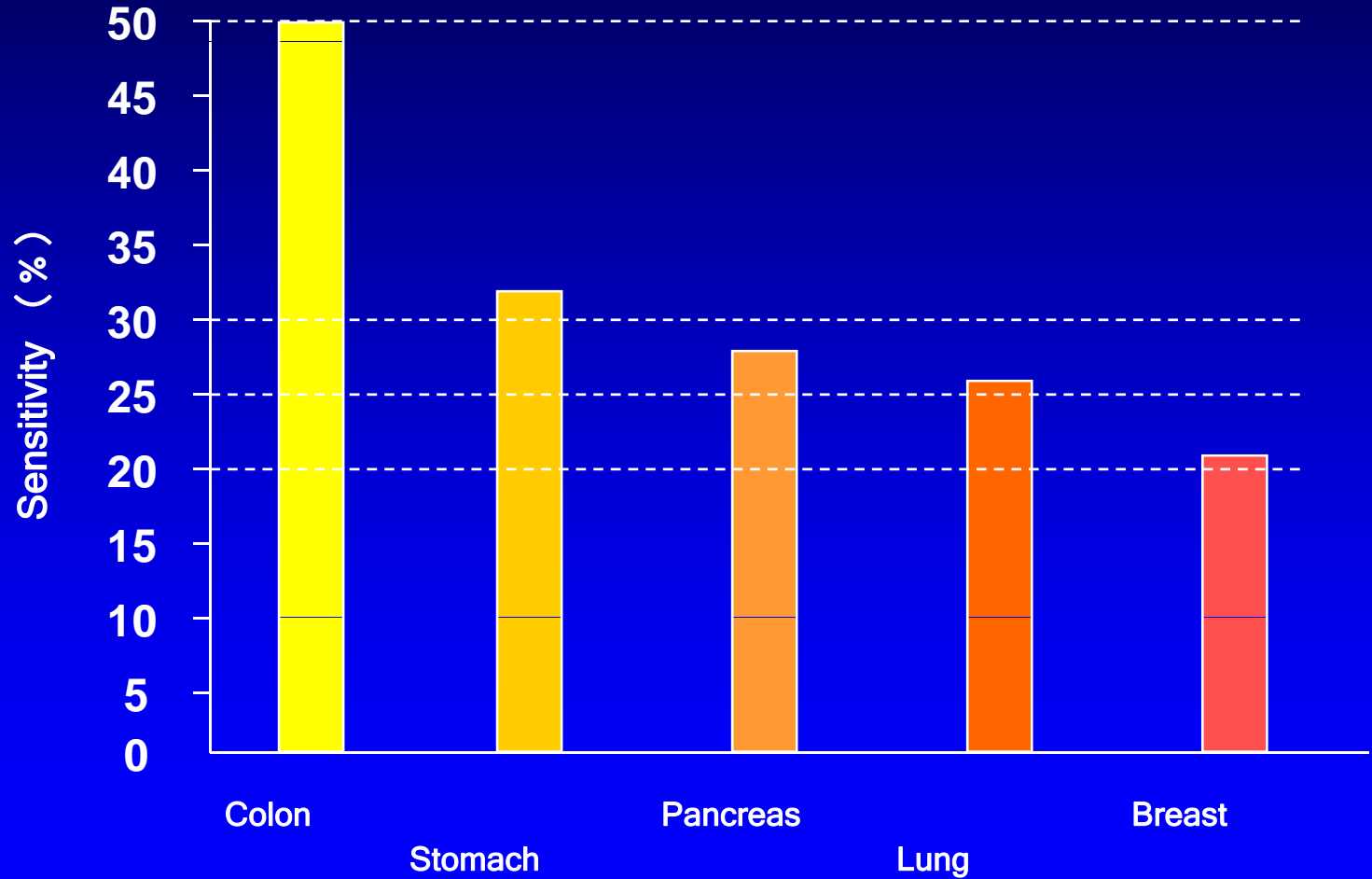
## Fecal markers

FOBT

DNA panel

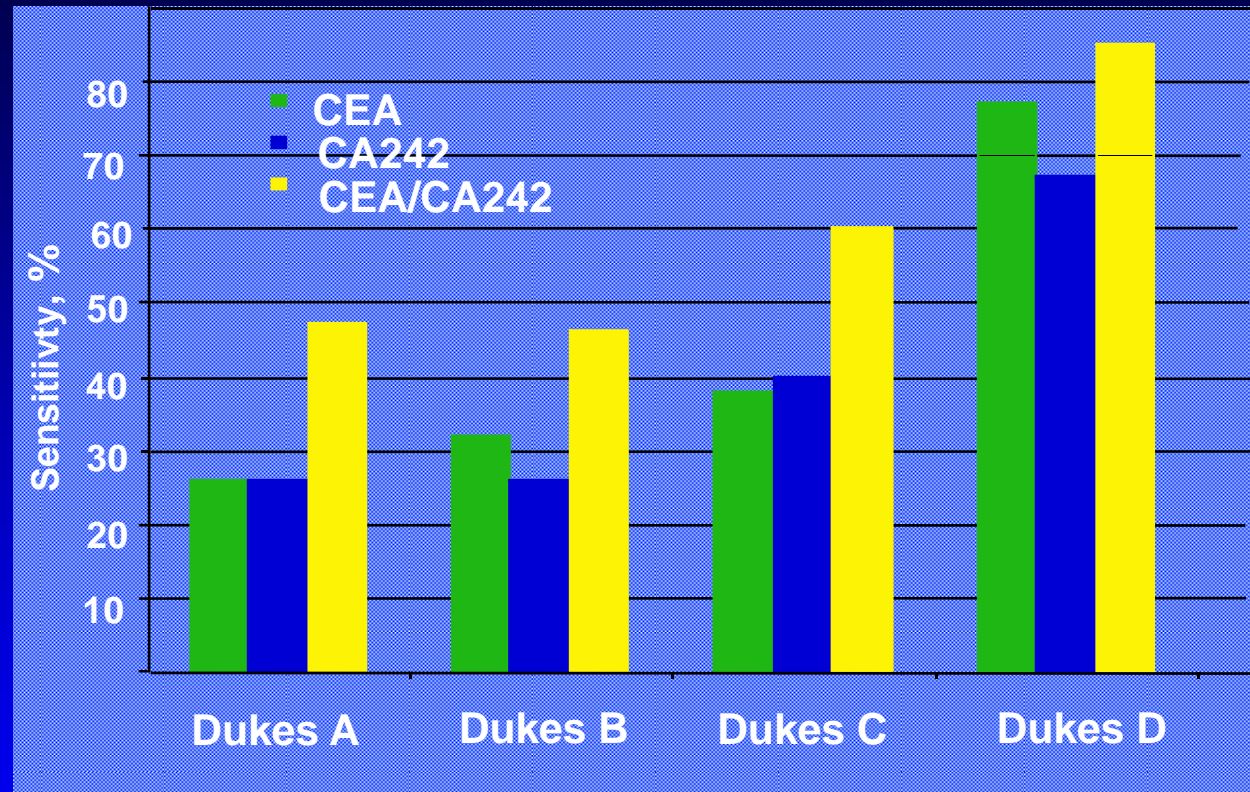
# Sensitivity of CEA

95% Specificity



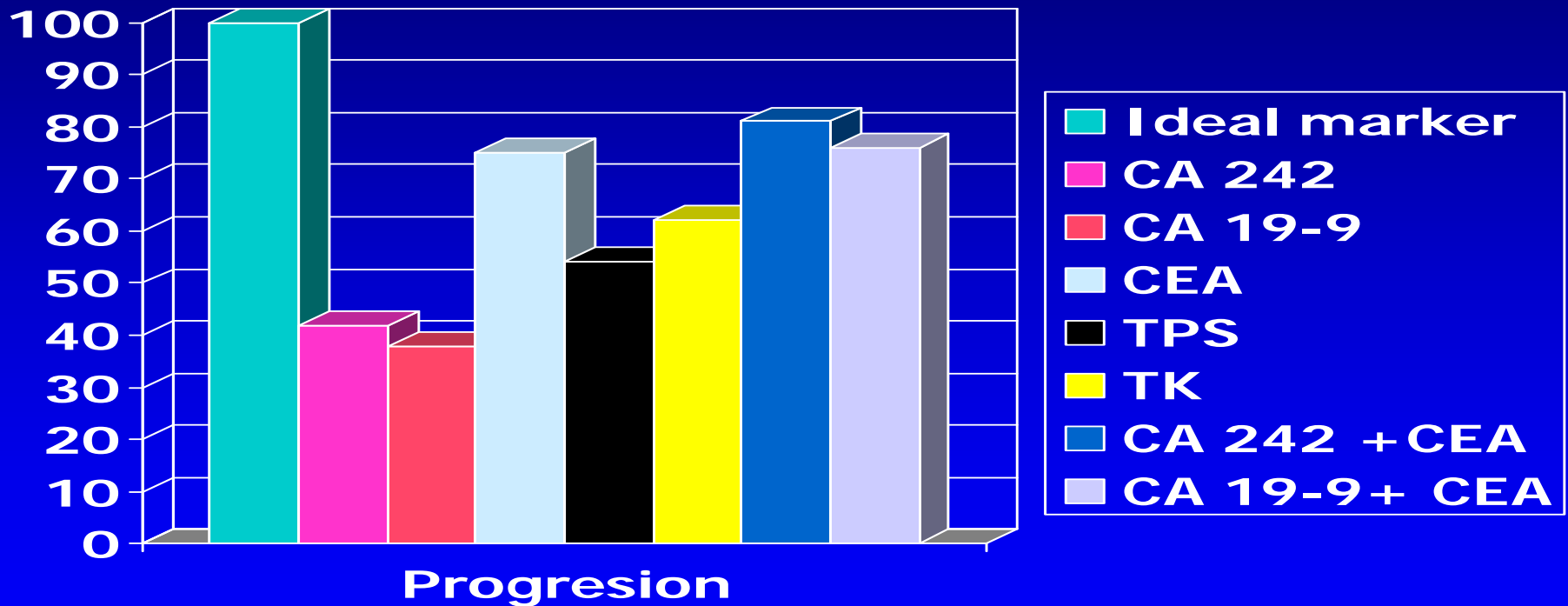
CEA

# CEA and CA242 in colorectal cancer



Carpelan-Holmström et al,  
1995

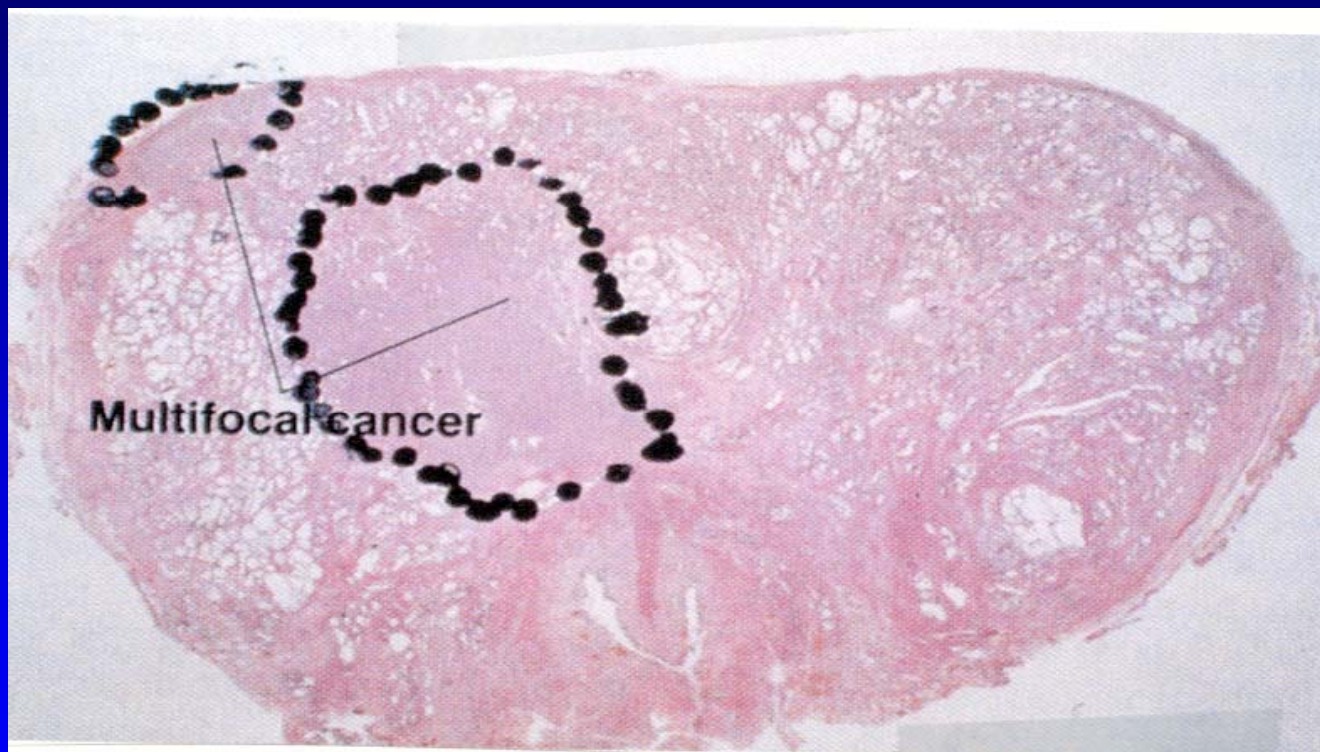
# Tumor marker sensitivity in patients with progressive colorectal cancer



## NACB and EGTM recommendations for use of tumor markers in colorectal cancer

- CEA or other serum markers or combinations can not be used in screening for CRC
- Assessing prognosis — high pre-op CEA levels predict adverse outcome (LOE III)
- FOBT for colorectal cancer screening
- CEA for surveillance following curative surgery — in combination with radiology, other markers and clinical history (LOE I)

# Prostate Cancer



## Diagnosing prostate cancer

- DRE
- Total PSA, Free PSA and ratio fPSA/PSA
- Prostate Biopsy — how effective?
  - Core needle biopsy - 25% confirm prostate cancer, 30-35% false negative
  - Core needle biopsy — expensive, painful, bleeding, risk of infection

# Biomarkers in prostate cancer

## Serum-based markers

PSA

fPSA

fPSA/PSA ratio

cPSA

## Molecular Urine markers

PCA3, GST, Telomerase activity

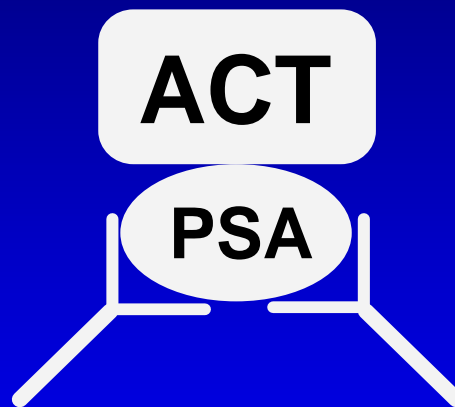
## Molecular/Gene tests

RT-PCR PSA, hK2, PMSA

PTEN

PSCA

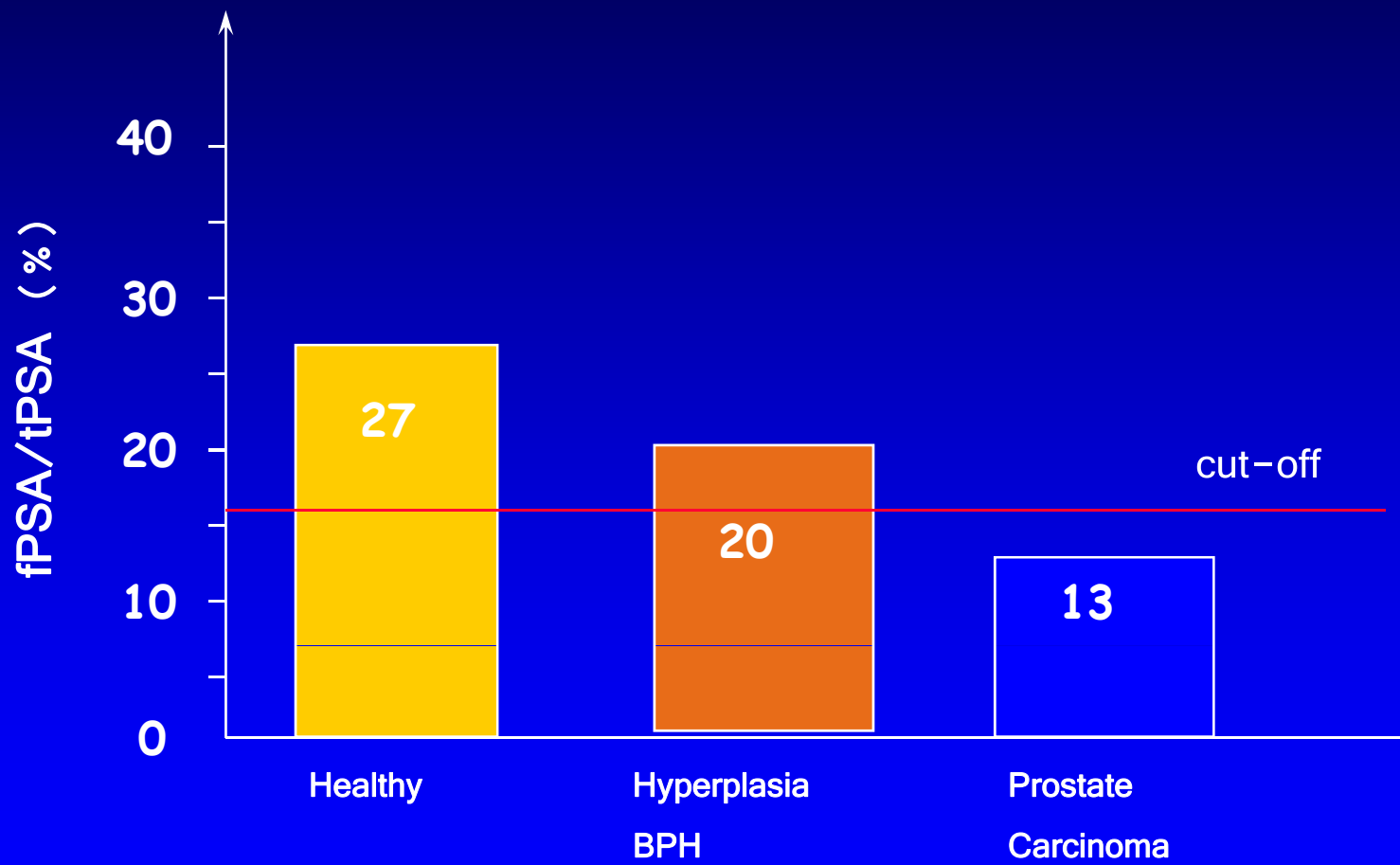
# Immunodetection of different forms of PSA



Christensson, A. et al (1990) *Eur. J. Biochem.* 194: 755-763.

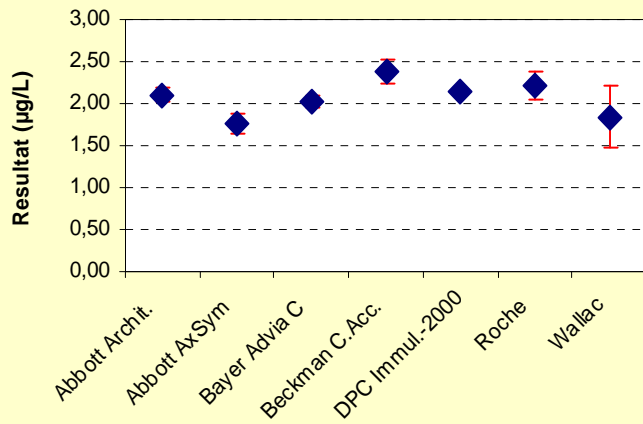
Lilja, H. et al. (1991) *Clin. Chem.* 37: 1618-1625

# fPSA/PSA discrimination

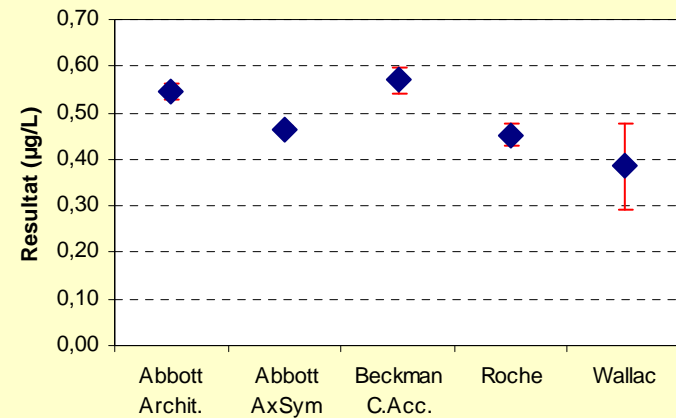


# Patient sample "2" assayed with different instruments, total and free PSA (average and SD)

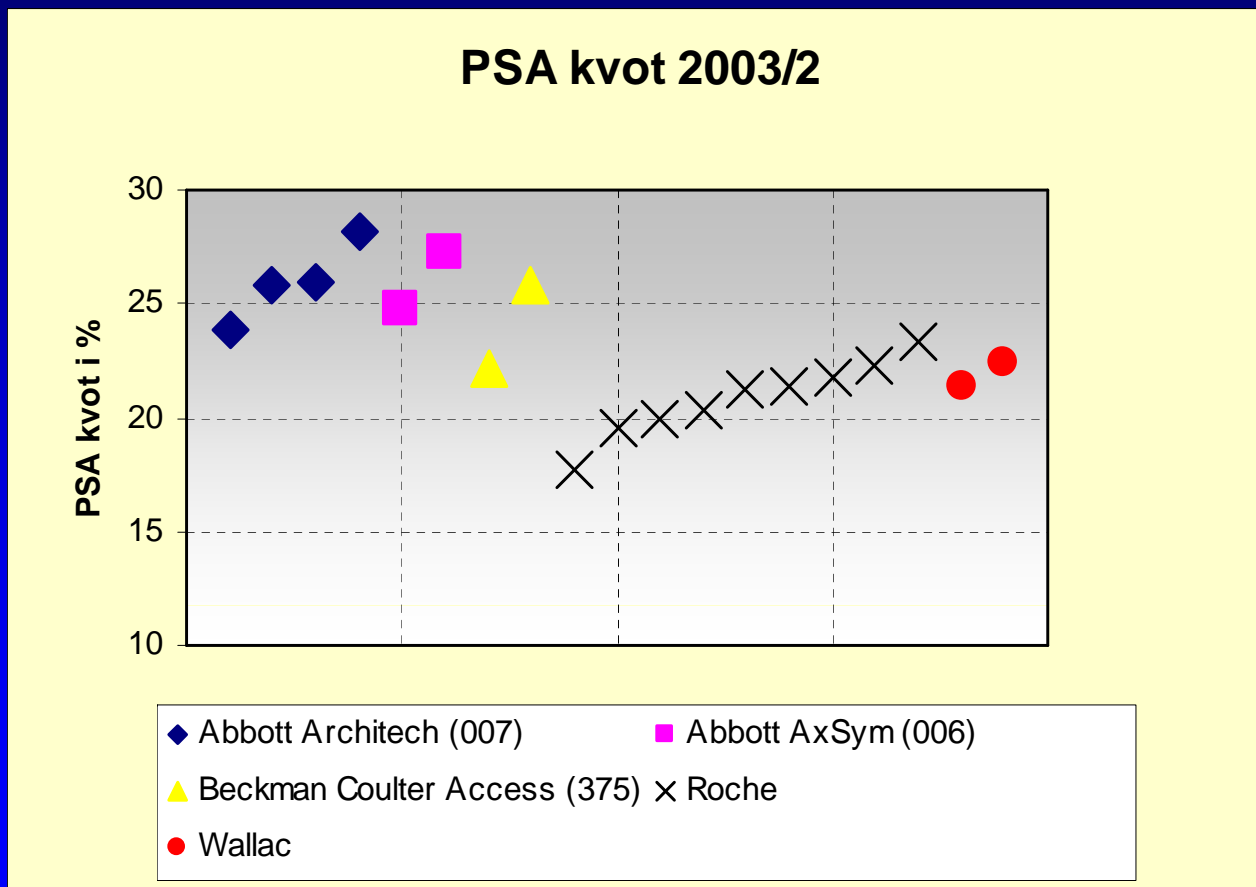
PSA total 2003/2



PSA fritt 2003/2



**FPSA/TPSA ratio; total PSA 2 ug/L, decision level F/T is 18 (%), F/T ratio automatically calculated when TPSA is in the range 3-10 ug/L. Ett exempel på PSA kvot där PSA total nivån är ca 2 µg/L.**



# NACB and EGTM recommendations for use of tumor markers in prostate cancer

- PSA and cPSA for screening/early detection of prostate cancer in combination with DRE (LOE I, III)
- PSA for staging, prognosis, management and monitoring of prostate cancer (LOE I, II, III)
- fPSA and fPSA/PSA ratio for differentiation of prostate cancer and BPH (LOE I)



# Lung cancer – histology and recommended biomarkers

Adenocarcinoma

– CYFRA, CEA

Squamous Cell Carcinoma

– SCC, CYFRA, CEA

Large Cell Carcinoma

– CYFRA, CEA

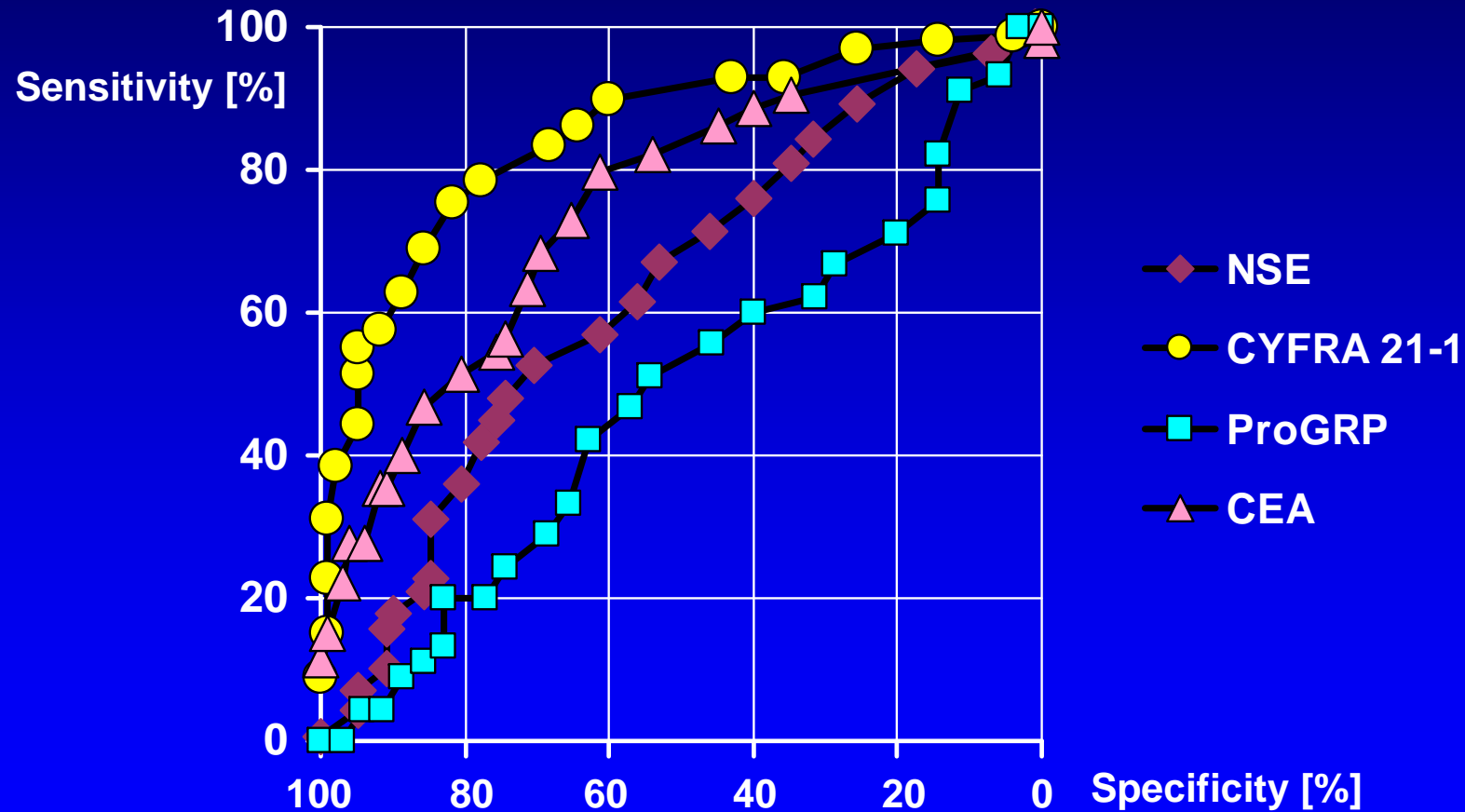
Small Cell Lung Carcinoma

– NSE

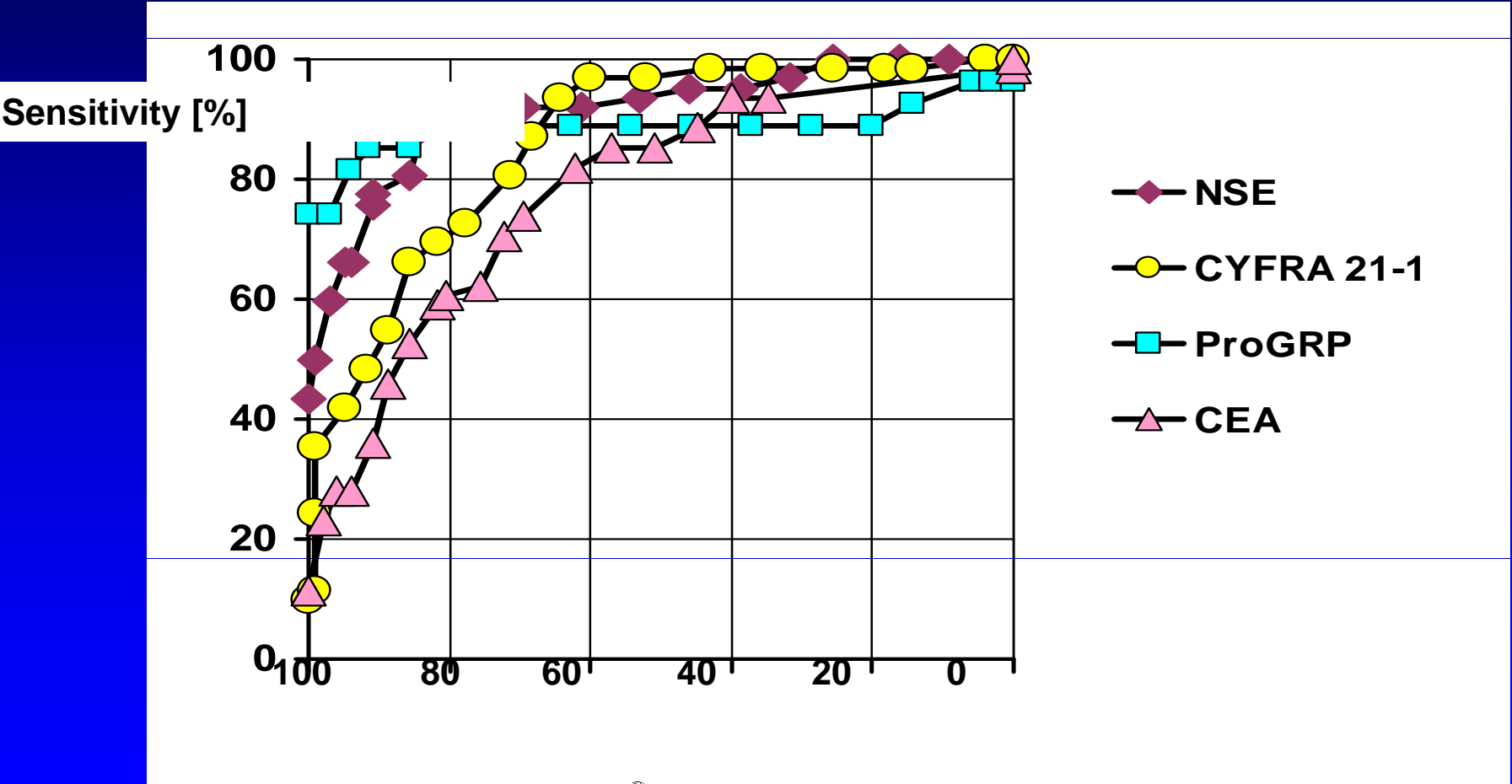
– ProGRP (Pro Gastrin Releasing Peptide)

# Benign Lung Diseases vs NSCLC

## ROC analysis



# Benign Lung Diseases versus SCLC ROC analysis



# Ovarian Cancer

## Female reproductive system

### Biomarkers

CA125

CA72-4

TPA/TPS

TATI

CASA



# Ovarian cancer – the greatest clinical challenge

Ovarian cancer has the highest fatality to case/ratio of all gynecologic cancers – 25 400 new cases and more 16000 deaths per year in US

## Mission:

- distinguish malignant from benign pelvic masses (differential diagnosis) prior to intervention
- early detection of recurrent disease during treatment
- identify patients early not responding to treatment

# Ovarian Cancer: Stage Distribution and Survival by Stage

<b>Stage</b>	<b>Distribution</b>	<b>Survival</b>
I	20-27%	73-93%
II	5-10%	45-70%
III	52-58%	21-37%
IV	11-17%	<5-25%

# Ovarian cancer - What modalities are available currently?

- Tumor markers
  - CA125
- Imaging
  - US, CT-scans and MRI
- Combination of imaging and tumor markers
  - CA125 and US
- Multiple marker assays
  - CA125 and CA72-4, CA125 and SMRP

# CA 125 Levels

- 80% of women with an epithelial ovarian cancer will have an elevated CA125 (cut point 35 U/ml in healthy control)
- Cut point is higher in premenopausal women and during menstruation and lower in women after menopause
- 50% of Stage I patients will have an elevated CA125
- CA125 is less often elevated in mucinous adenocarcinomas and germ cell tumors

# Sensitivity for Ovarian cancer according to Histology and Stage

Type/Stage	CA 72-4	CA 125
<b>Serous</b>	<b>56 %</b>	<b>70 %</b>
<b>Endometrioid</b>	<b>64 %</b>	<b>73 %</b>
<b>Mucinous</b>	<b>69 %</b>	<b>46 %</b>
<b>Others</b>	<b>88 %</b>	<b>88 %</b>
<b>1</b>	<b>47 %</b>	<b>50 %</b>
<b>2</b>	<b>57 %</b>	<b>64 %</b>
<b>3</b>	<b>62 %</b>	<b>90 %</b>
<b>4</b>	<b>67 %</b>	<b>&gt;90 %</b>

# Ovarian Cancer - Screening Tool

- accurate tumor markers
  - Sensitivity > 75 %
  - Specificity > 99.6 %
  - PPV > 10 %
- safe, simple, inexpensive and tolerable

Future directions

Combination of imaging and tumor markers?

# Additional biomarkers in ovarian cancer

Gene expression and histochemical analyses have identified a number of possible biomarkers

- Mucins (MUC1, DF3, CA19-9, CA72-4)
- Kallikreins (hk4, hK6, hK8, hK10)
- Mesothelin
- Antileukoproteinase 1 (ALP1)
- Osteopontin
- Claudin 3/Claudin 4
- Ep-CAM
- VEGF
- HER-2
- Inhibin
- Activin
  
- **HE4**
  - **One of the most commonly up-regulated biomarker in ovarian cancer**
  - **Up-regulated - mRNA and protein level in early and late stage disease**

# Biochemistry of HE4

## HE4 - Human Epididymis secretory protein 4

- Member of the WAP-gene family of protease inhibitors (*WFDC-gene family*)
- Characterized by a 50 amino acid sequence, 8 highly conserved cysteine residues that form 4 disulphide bridges.

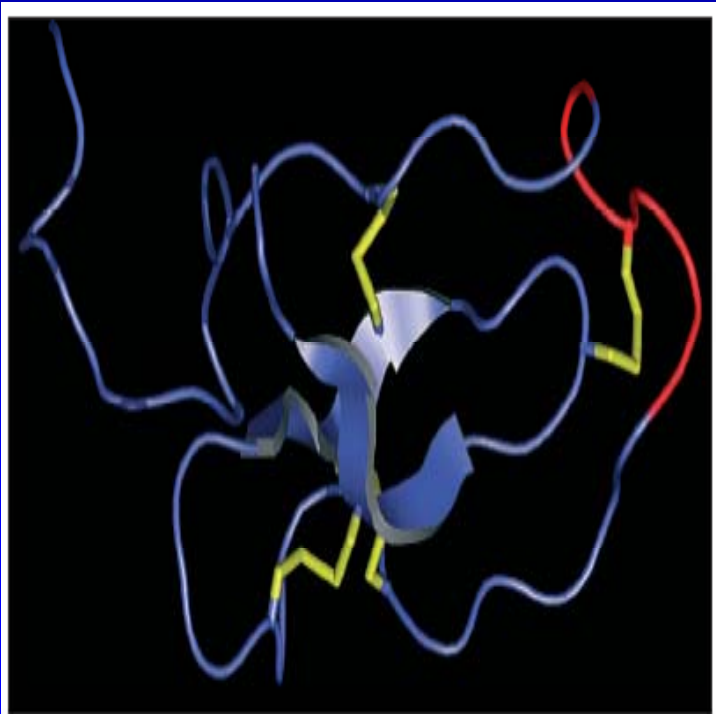
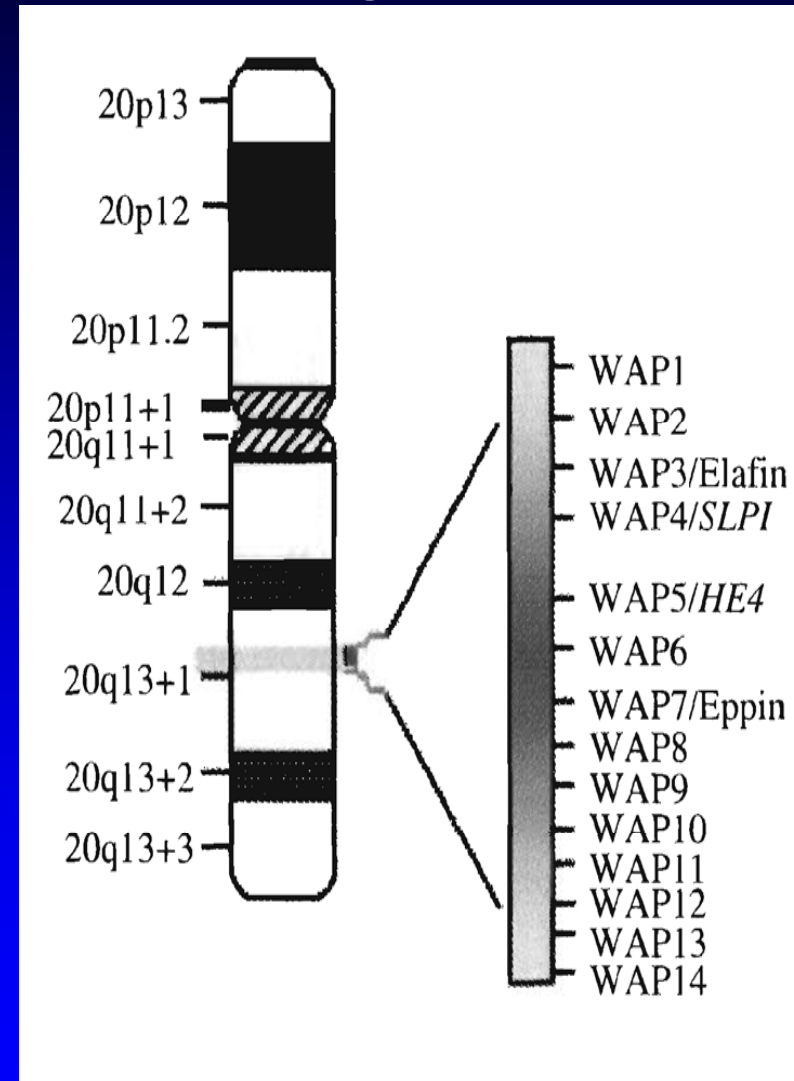


Figure 1: Typical structure of WAP motif

## WFDC gene locus



# Cross-validated Estimates of Sensitivity

Average from Leave-One-Out Analysis  Marker Combination	Benign vs. Ovarian Cancer: Sensitivity at		
	90% Specificity	95% Specificity	98% Specificity
CA125	61.2%	<b>43.3%</b>	23.9%
<b>HE4</b>	77.6%	<b>72.9%</b>	64.2%
<b>CA125 + HE4</b>	<b>80.7%</b>	<b>76.4%</b>	71.6%
<b>CA125 + HE4 + SMRP</b>	<b>80.6%</b>	<b>74.7%</b>	<b>71.7%</b>
<b>CA125 + HE4 + CA72-4</b>	<b>82.1%</b>	<b>78.8%</b>	<b>71.5%</b>

Moore et al. 2007

# Cross-validated Estimates of Sensitivity for Stage I

Average from Leave-One-Out Analysis Marker Combination	Benign vs Stage I Ovarian Cancer Assay Sensitivity (%)		
	90% Specificity	95% Specificity	98% Specificity
CA125	23.1%	15.1%	7.7%
HE4	46.2%	45.9%	30.8%
SMRP	30.8%	30.3%	15.4%
CA72-4	23.2%	23.1%	23.1%
CA125 + HE4	46.1%	39.5%	38.4%
CA125 + SMRP	38.5%	30.6%	23.0%
CA125 + CA72-4	30.7%	30.0%	23.1%
CA125 + HE4 + CA72-4	46.1%	38.4%	38.4%
CA125 + HE4 + SMRP + CA72-4	53.4%	38.4%	38.4%

# Conclusions

- **Laboratory tests will continue to be a main part of the war on cancer**
- **Tumor markers will assist in screening, diagnosing and monitoring cancer**
- **Tumor marker determinations, applied in a proper way in the appropriate situation, are a powerful support to the clinicians**