An addition to Tumor markers for Epithelial Ovarian Cancer

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I have no relevant financial relationships to disclose.

Tumor marker (1)

- ***Definition:*** Molecules or substances produced by malignant tumors that enter the circulation in detectable amounts, which indicate the likely presence of cancer or provide information about its behavior.

✓ Enzymes, hormones, receptors, growth factors, biologic response modifiers, and glycoconjugates

✓ **Tumor associated markers** rather than tumor specific

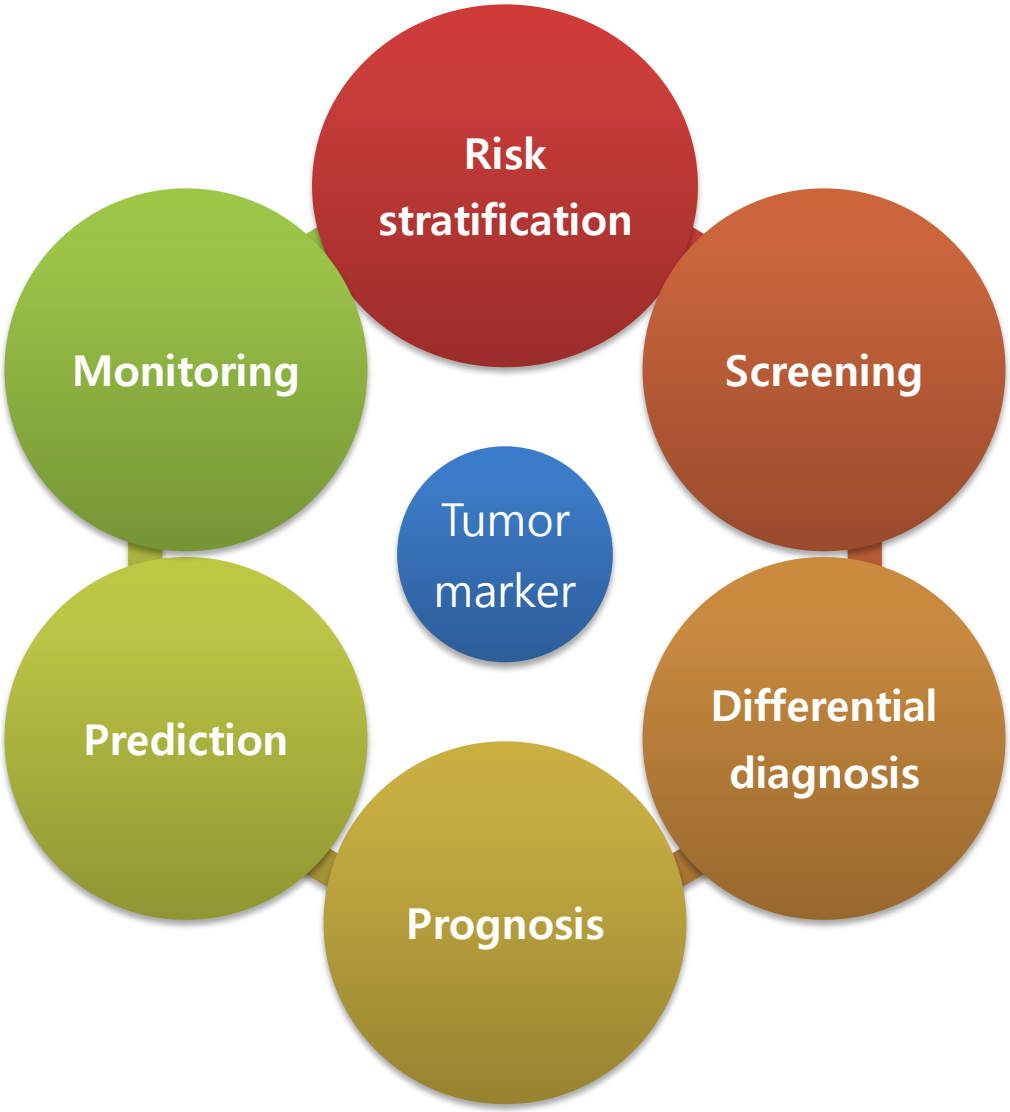
Tumor marker (2)

- Tumor markers contribute to DDx, but are not themselves diagnostic.

- Only a few tumor markers have been validated for clinical use: **Cancer antigen 125**

- Potential tumor markers
  - ✓ A substantial number of substances have been investigated over the past decade
  - ✓ The list is continually growing owing to new technology employed in biomarker discovery.

Potential Uses of Tumor Markers



Classification of Tumor marker

Diagnostic Tumor marker

Prognostic Tumor marker

Provide evidence about the patient's overall disease outcome independent of any specific intervention

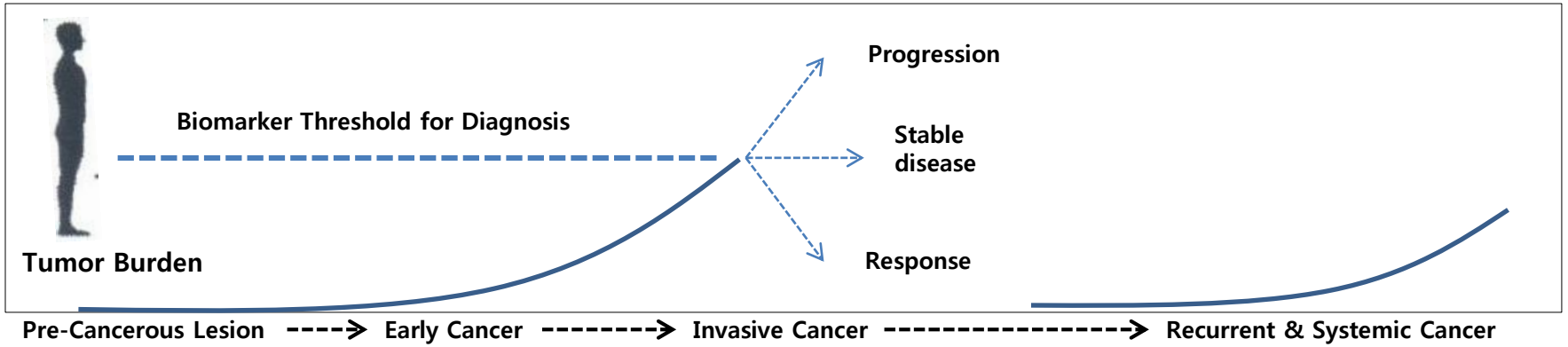
Predictive Tumor marker

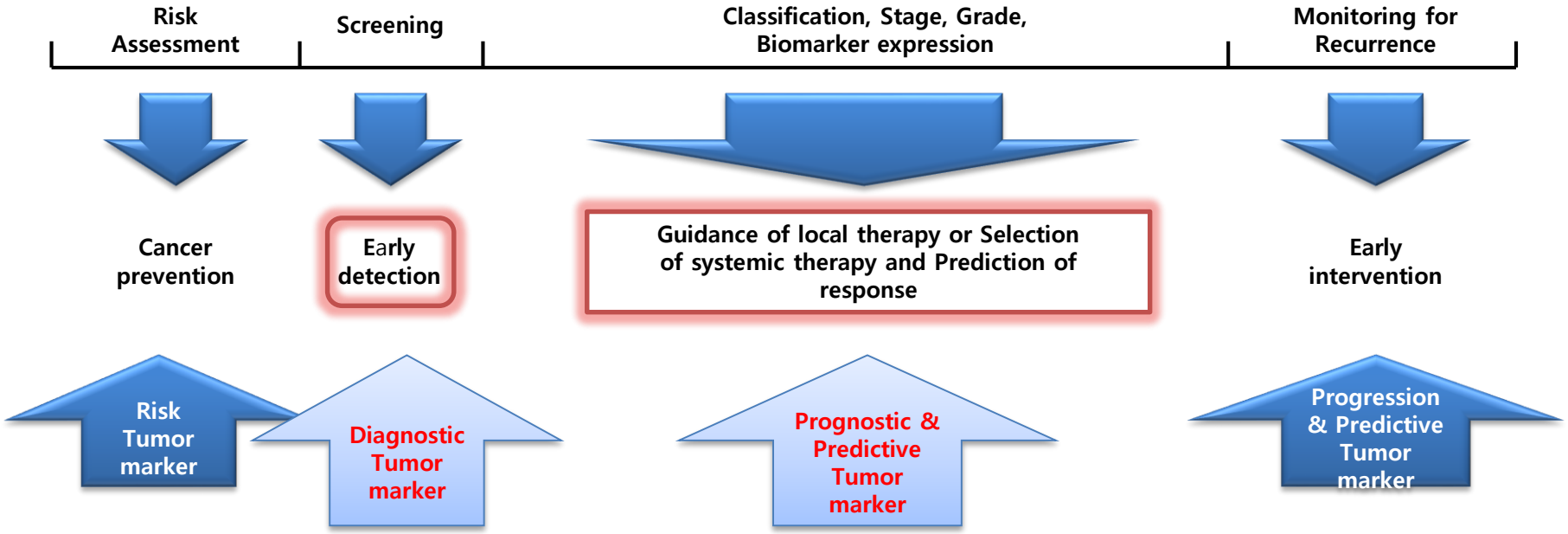
Provide evidence about the probability of benefit or toxicity from a specific intervention

- Conventional treatment
- Molecular targeted therapy

Patient selection
Efficacy prediction
Optimal dosing (Toxicity)

Schematic Representation of Tumor & Tumor marker





Parameters of Tumor Marker Assays

Tumor Marker Result	Positive	Negative
Positive	A	B
	(True Positives)	(False positives)
Negative	C	D
	(False Positives)	(True Negatives)

Sensitivity=True positives/ All with tumor=A/A+C
Specificity=True negatives/All tumor free=D/D+B
Positive predictive value (PPV)=True positives/ All with positive tumor-marker result=A/A+B

Why We Should Try to Predict Ovarian Cancer Earlier?

1. Current therapies are effective for early disease
   - in FIGO stage Ⅰ/Ⅱ : 73-93%
   - in FIGO stage Ⅲ/IV : only 30%
   - ✓ However, 70% of patients : detected in advanced stage

   (SEER Cancer Statistics Review, NCI, USA, 2011).

2. Initial surgery is common in local private, not by gynecologic oncologists (Cancer 2007; 109:2031-42)

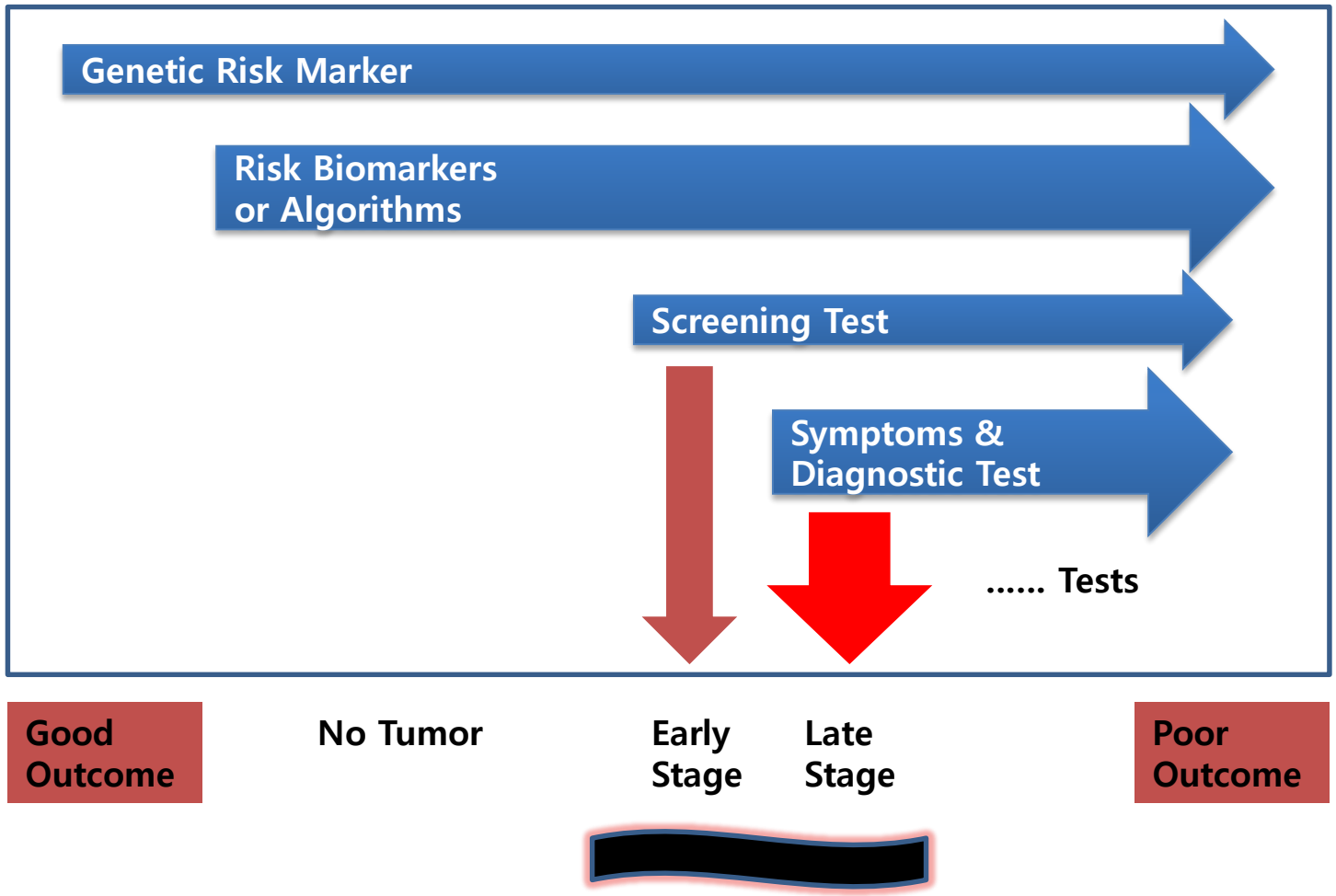
Requirements for Early Detection

- Effective screening methods have not been established in ovarian cancer, elusive! ***"No Standard Screening Test"***

- **Low prevalence** of ovarian cancer even in postmenopausal population (1:10,000, Korea)
  - **High sensitivity** (>75%) is required for early stage (ideally, pre-clinical disease)
  - **Extraordinarily high specificity** (>99.9%) is also needed to achieve a PPV of 100%

- For **cost effectiveness** & **acceptable risk**

Lead Time & Outcome



Needs for Early Detection of Ovarian Ca.

1. Tumors must arise from single clones of cells and metastatic disease should develop by progression from clinically detectable lesions

2. Length of time of stage I must be sufficiently long to permit cost-effective screening at practical intervals

   – A model of natural Hx. Of OC by Brown & Palmer
   # 4 years for in situ, stage I/II, 1 year for stage III/IV

   – Skates et al, analyzed CA125 in U.K (28 ov. ca / 22,000 women)
   # Preclinical ov ca: 1.9 ± 0.4 years

   → ***So, if cancers remained in stage I for half of this interval, annual screening should be effective***.

PlosS. Med 2009 6(7) e1000114
J Clin Epidemiol 1991; 44:365

CA 125 in EOC

- **The single most sensitive and specific marker**
- **CA125 elevation:**
  - Most than 80% of ovarian cancer patients
  - Only 1% of the general population

Proportion of positive CA125 test (usually, >35 U/mL)					
FIGO stage	Stage Ⅰ	Stage Ⅱ	Stage Ⅲ	Stage Ⅳ	TOTAL
	50.0%	90.0%	92.1%	93.9%	85.1%
Histologic Type	Serous	Mucinous	Endometrioid	Clear cell	Undiff.
	80%	69%	75%	78%	88%

→ 62~86% Sensitivity and 95~99% Specificity *limitation*

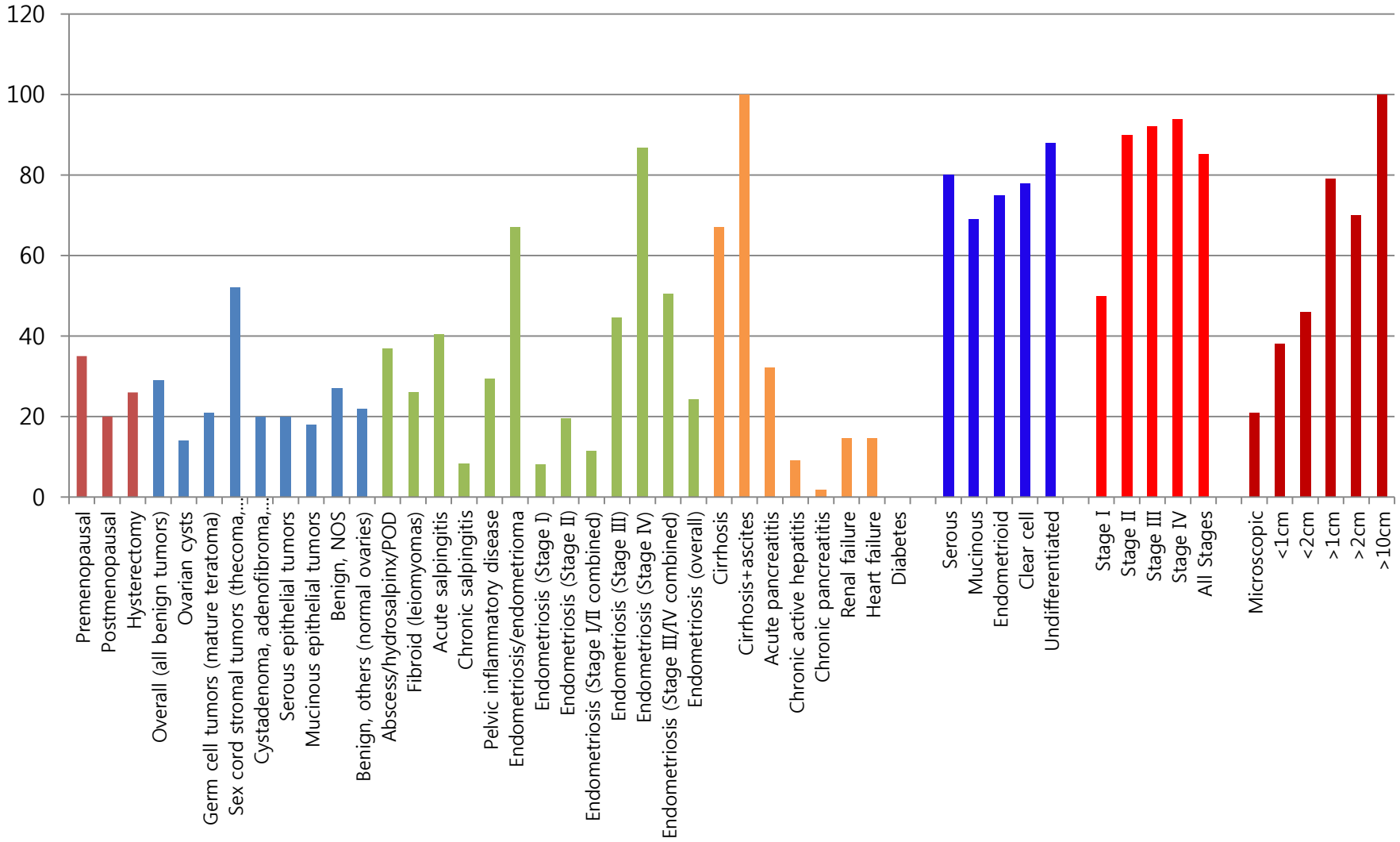
Petricoin EF, et al. Lancet 2002; Bast RC Jr, et al. Cancer Treat Res 2002

Expression of CA 125 on derivatives of the coelomic epithelium

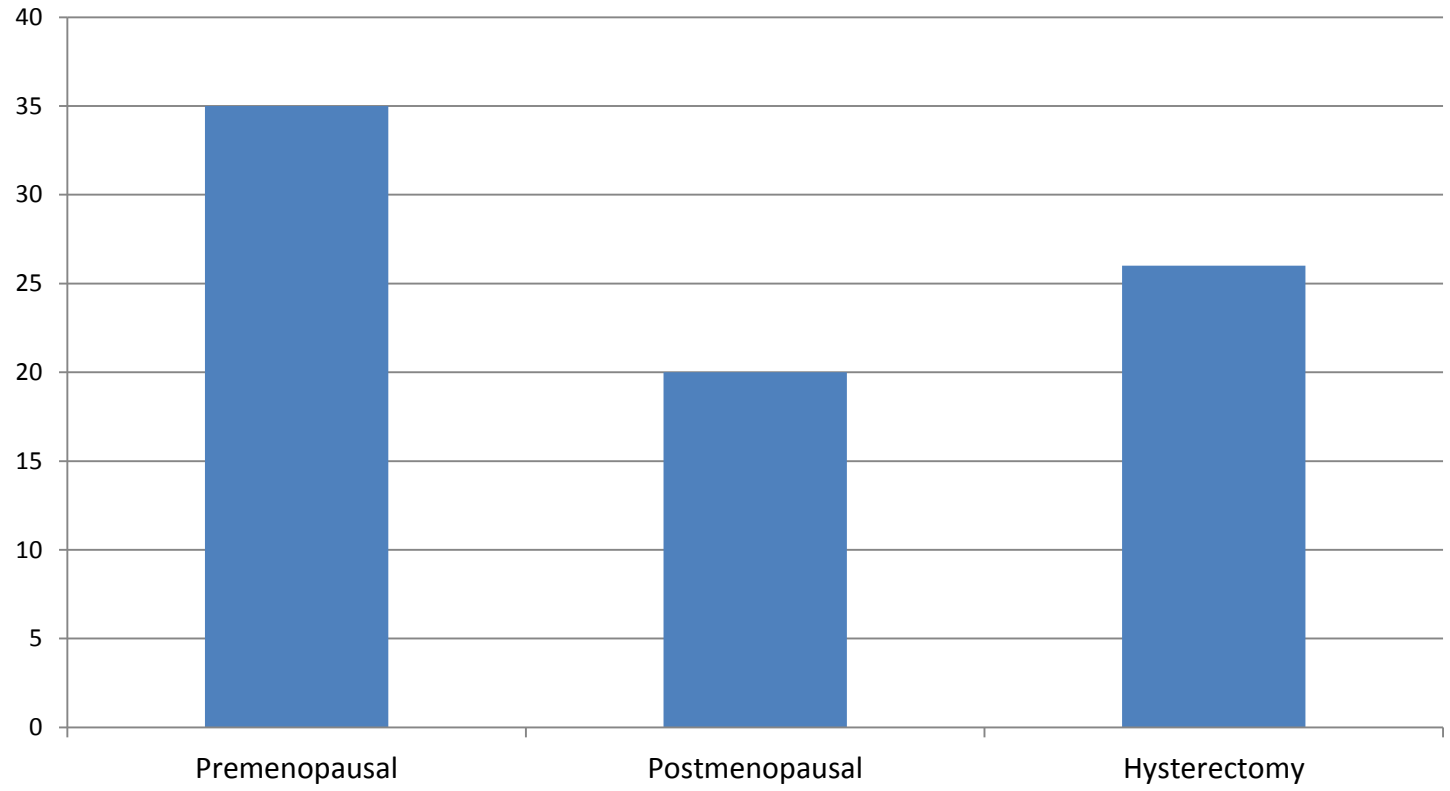
Fetus	Adult	Neoplasms
Mesothelium		
Peritoneum	Peritoneum	
Pericardium	Pericardium	
Pleura	Pleura	Mesothelioma
Mullerian epithelium		
Fallopian tube	Fallopian tube	Adenocarcinoma
Endometrium	Endometrium	Adenocarcinoma, MMMT
Cervix	Cervix	Adenocarcinoma
Ovarian epithelium		
-	-[a]	Serous, endometrioid, clear cell tumor
Noncoelomic derivatives		
Amnion	-	
-	-	Adenocarcinoma of lung and breast

Kabawat SE et al., Int J Gynecol Pathol. 1983;2(3):275-85
[a]Epithelial inclusion cysts and papillary excrescences

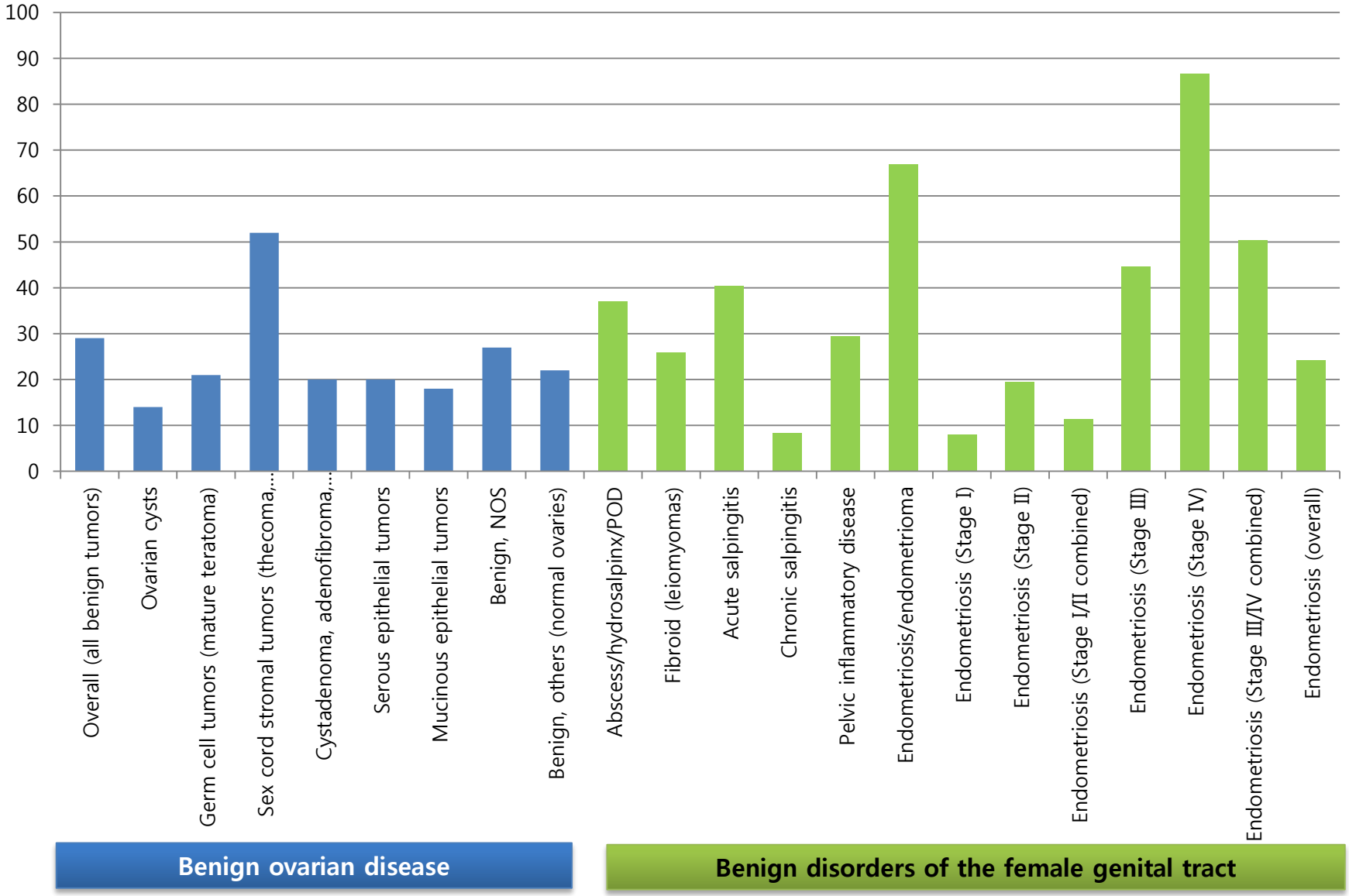
CA 125 Elevations in Benign Disorders and Ovarian Cancer



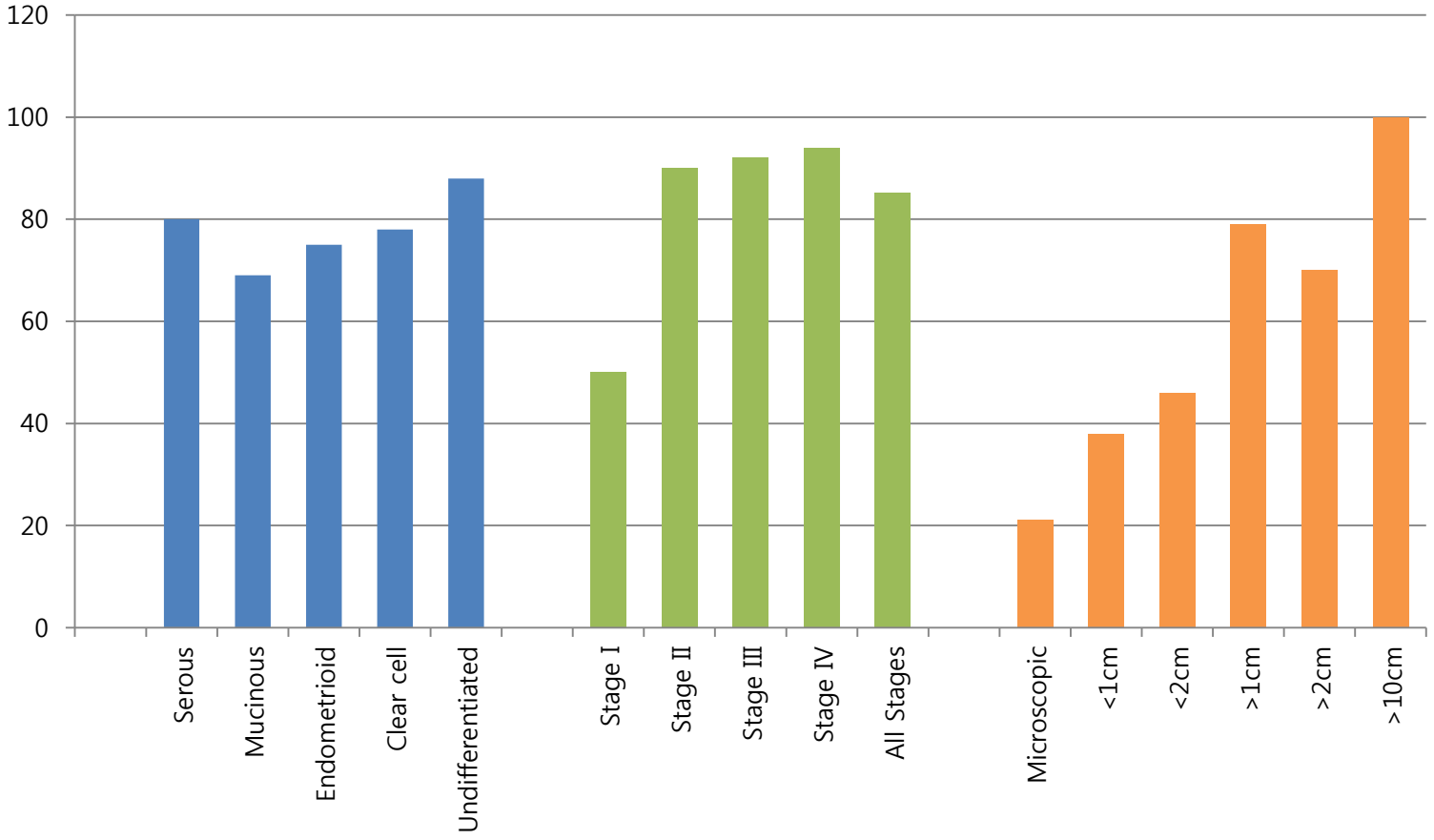
CA 125 Elevations in Healthy Women



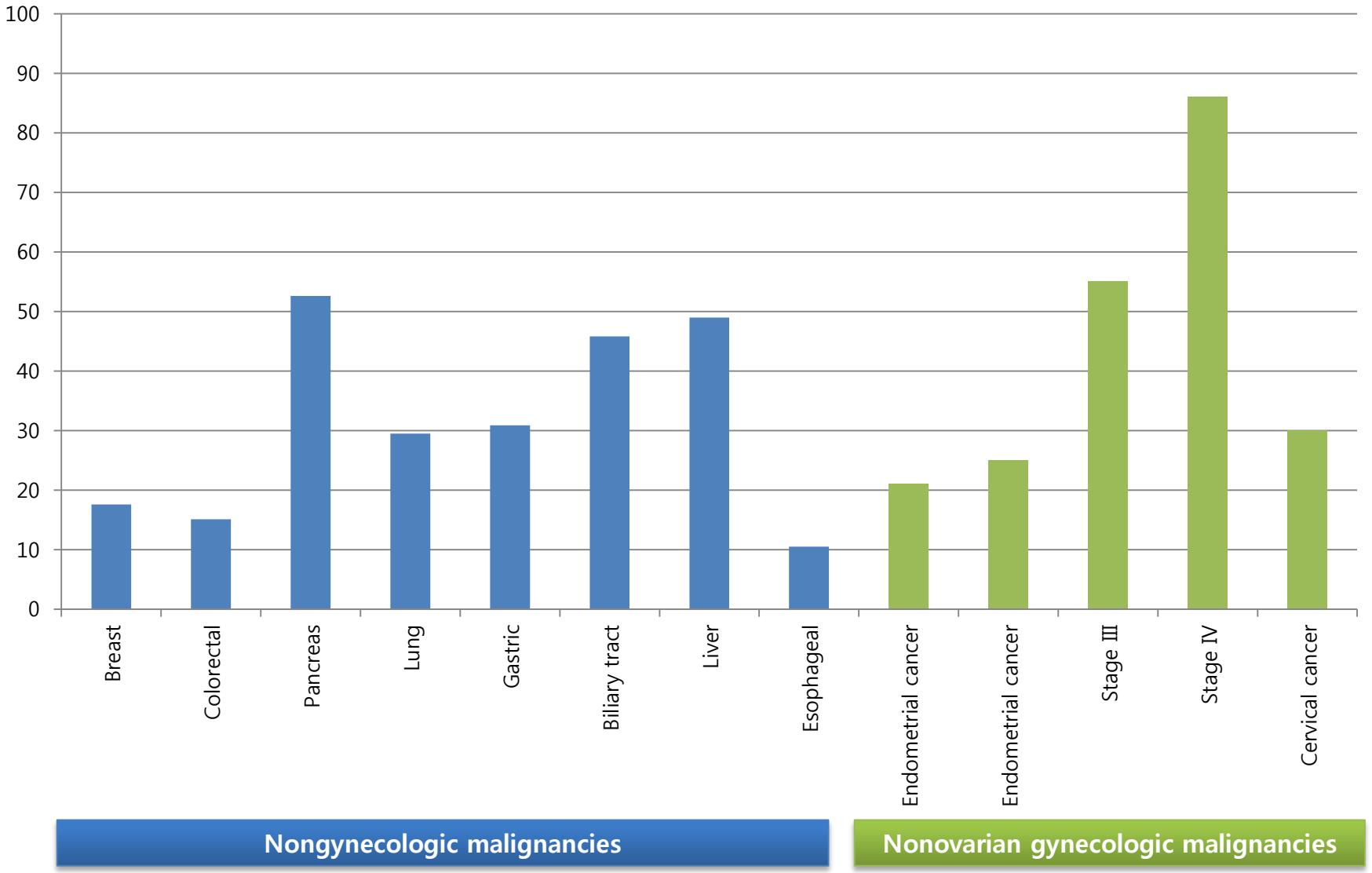
CA 125 Elevations in Benign Diseases



CA 125 Elevations in Ovarian Cancer



CA 125 Elevations in Nonovarian Malignancies



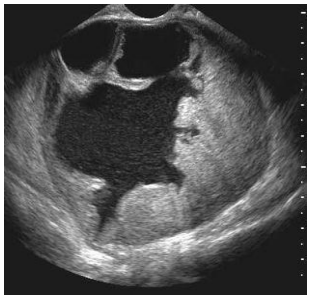
Screening

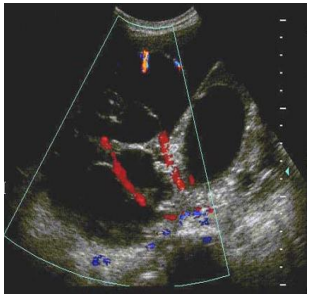
1940 Cervical cancer
1960 Breast cancer
1970 Lung cancer
1975 Colorectal cancer
1990 Prostate & Ovarian cancer

***Screening for OC continues to be investigated in the research setting with CA125 the only tumor marker currently being explored in large trials.**

Ultrasonography in EOC

- **Independent Prognostic Ultrasound Variables**
  - Maximum diameter of lesion
  - Ascites
  - Blood flow within a solid papillary projection
  - Presence of an entirely solid tumor
  - Maximal diameter of solid component
  - Irregular internal cyst walls
  - Acoustic shadows
  - Color score of intramural blood flow





→ 93% Sensitivity and 76% Specificity in Adnexal Mass (TVS only)

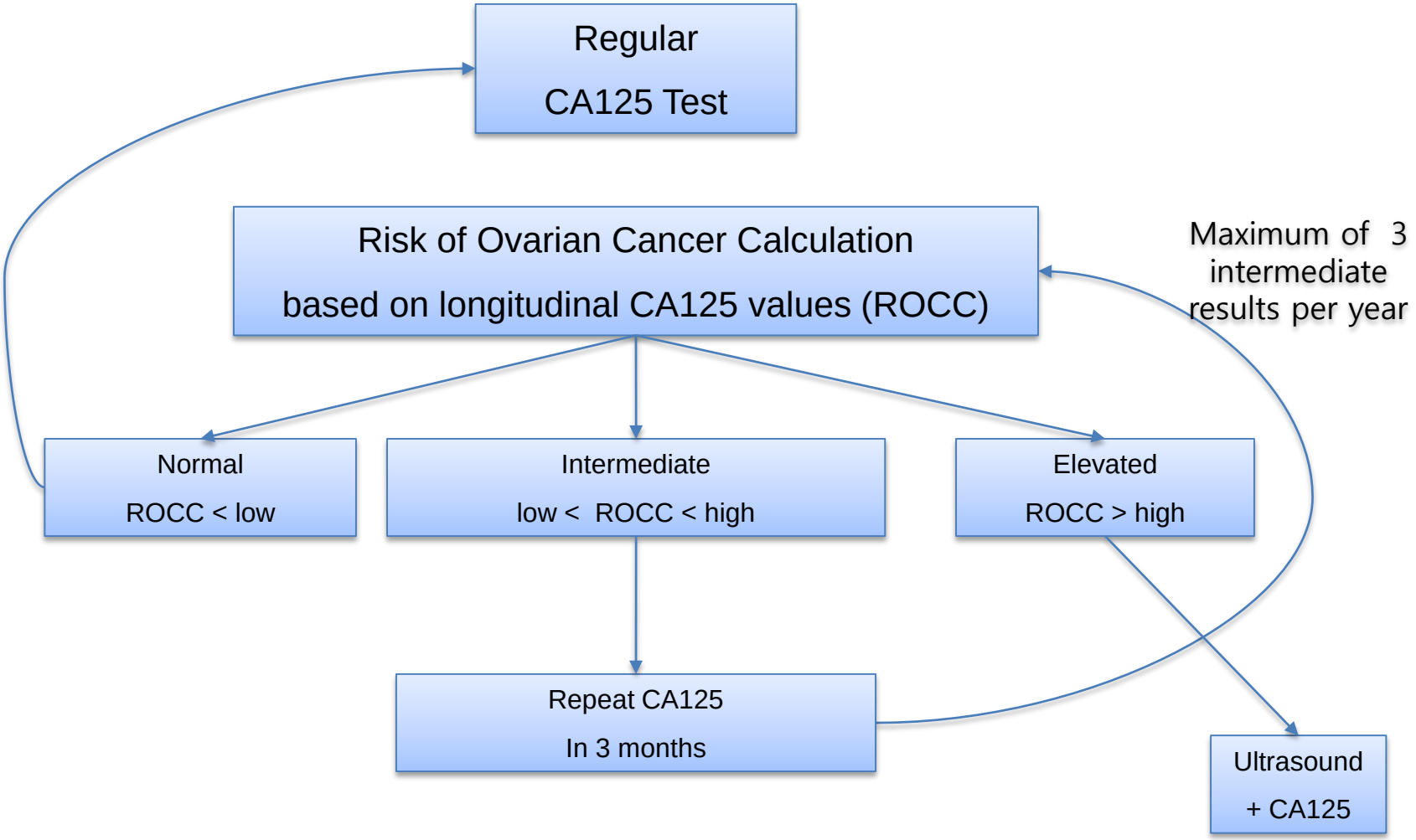
99.9% specificity and 4 operations for each OC. (CA125 and TVS)

Risk of Ovarian Cancer Algorithm (ROCA)

- Developed by Dr. Steven Skates
- Based on **age, menopausal status** and **change of CA-125 over time**

- **ROCA**
  - Improves sensitivity
    :By examining change of CA-125 over time
  - Improves specificity
    :Each woman establishes her own baseline

ROCA: Risk of Ovarian Cancer Algorithm



Skates JCO 2003

Large trials on OC screening

Kentucky Screening study

- Single arm annual Ultrasound screening
- 25,327 women
- Sensitivity : 81% (primary EOC) & 76% (primary invasive OC)
- 82% of primary EOC: early stage (I/II)
- 5 yr survival : 74.8% (primary invasive OC, screen +, interval ca) vs 53.7% (not screened)

Japanese Shizuoka Cohort Study of Ovarian Cancer Screening

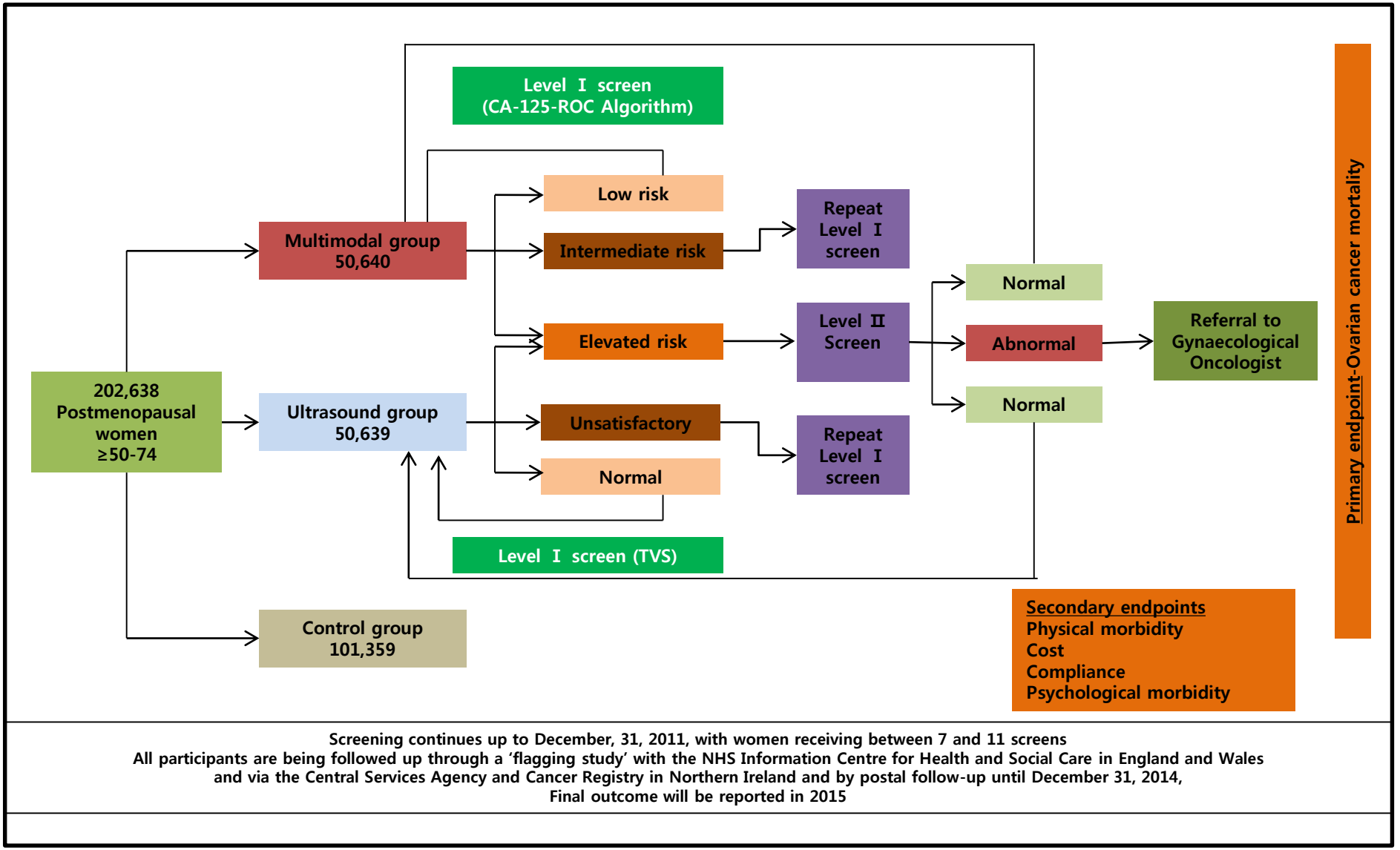
- 82,487 low-risk postmenopausal women
- Annual ultrasound and CA125
- Sensitivity: 77.1%, Specificity: 99.9%
- OC detection rate: 0.31 per1000 women at prevalent screen
  0.38-0.74 per 1000 women at subsequent screen
- Stage I OC: 63% (screened group) vs 38% (control group)

US Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial

- 78,237 women aged 55-74 years+34,202 women randomized to OCS
- CA 125 and ultrasound for 3 years and CA 125 alone for 2 years
- F/U median 12.4 years
- 4.7% women had an abnormal scan and 1.4% an abnormal CA 125
- PPV of invasive ca: CA125(3.7%), ultrasound (1%), both (23.5)
- Stage I/II: 28%

UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS)

- 202,638 postmenopausal women aged 50-74 years
- Annual ultrasound or a multimodal strategy in a 2:1:1
- ROC algorithm (absolute value of CA 125 and serial levels over time
- Intermediate risk: repeat CA125 in 12 weeks
- Elevated risk: ultrasound + repeat CA 125 in 6weeks



The United Kingdom Collaborative Trial of Ovarian Cancer Screening(UKCTOCS). Women in the trial, based on the risk of ovarian cancer (ROC) value, are triaged into **low risk** (ROC, <1 / 3,500), and returned to annual screening with the next blood test in one year; **intermediate risk** (<1 / 1,000 and <1 3,500) with a repeat CA-125 in 12 weeks; and **elevated risk** (>1 / 1,000) with Level Ⅱ screen (CA-125 and TVS) scheduled in 6-8 weeks, with earlier screens arranged where there was a high index of suspicion. Those with persistent abnormalities on Level Ⅱ screen are referred to a gynecologic oncologist. The screening protocol has been described in detail elsewhere. Roc, risk of ovarian cancer; TVS.

OC screening in the familial context

UK Familial Ovarian Cancer Screening Study(UKFOCSS)	• Prospective study • 5700 women • Annual ultrasound & CA 125 (ROC algorithm)
Cancer Genetics Network and GOG	• **ROC algorithm** • **3-4 monthly**
US based Cancer Genetics Network (CGN)	• ROC algorithm • 2343 high-risk women • 3 monthly • PPV 13%

Additional Markers

* To identify either a better marker or a panel of markers in screening
* Most of studies used clinical samples rather than preclinical samples.

Multivariate Serum Protein Biomarkers (1)

Development and preliminary evaluation of a multivariate index assay for OC. Amonkar et al. 2009

11-analyte profile: CA-125, CA 19-9, EGF-R, C-reactive protein, Myoglobin, Apolipoprotein A1, Apolipoprotein CIII, MIP-1a, IL-6, IL-18, Tenascin C

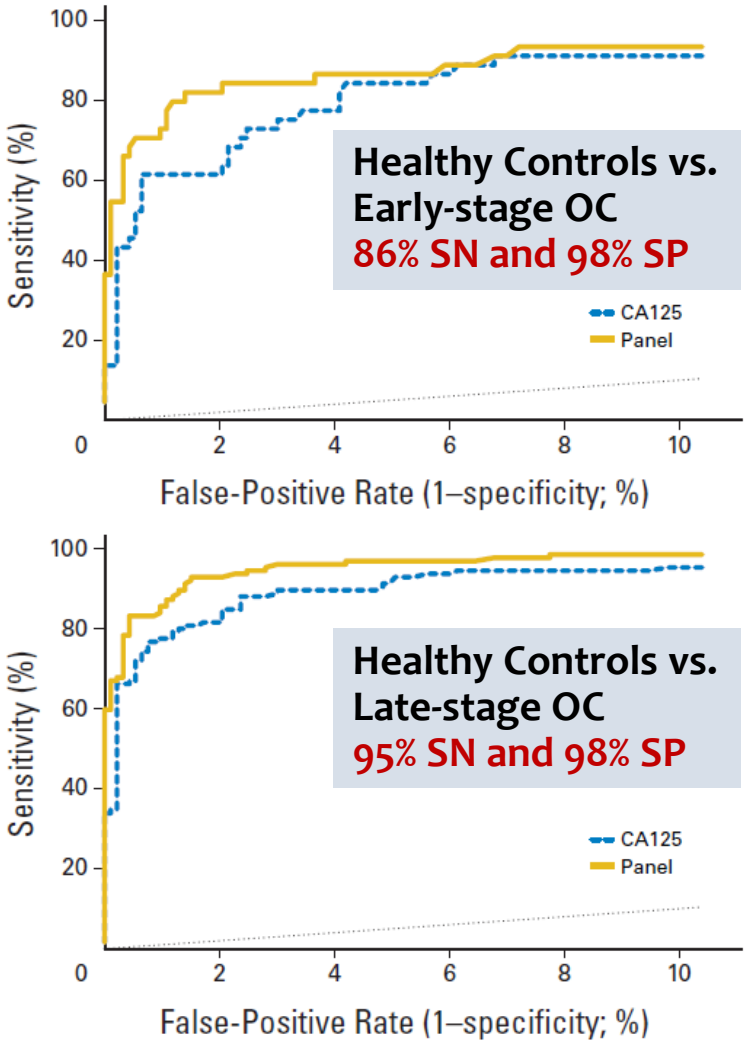
Method	Sensitivity	95% CI	Specificity	95% CI
All Samples* CA-125>35 IU/ml	**94.9%**	90.5–97.6	**58.6%**	51.9–65.6
Stage I only# CA-125>35 IU/ml	**88.5%**	77.8–95.3	**58.6%**	51.9–65.6
Final 11-analyte classifier¶	**91.3%**	84.2–95.5	**88.5%**	81.4–93.2

CA-125 only : 94.9% sensitivity and 58.6% specificity
11-analyte profile : 91.3% sensitivity and 88.5% specificity

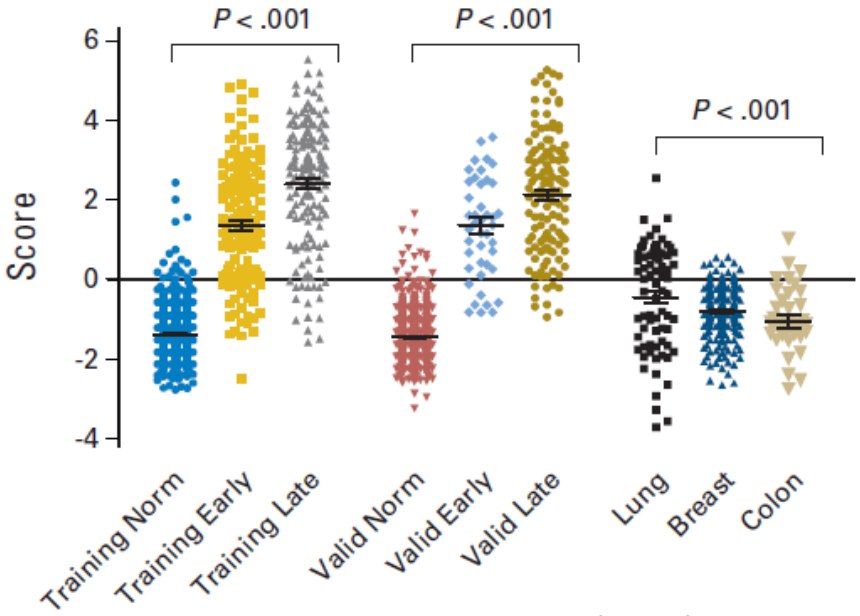
PLoS ONE 2009;4(2): e4599

Multivariate Serum Protein Biomarkers (3)

Yurkovetsky et al 2010



◀ CA125, HE4, CEA, VCAM-1



J Clin Oncol 2010;28:2159-2166

OVA1

- Composed with **CA-125, TTR(prealbumin), ApoA1, Beta2M and TFR**
  - The levels of these 5 proteins are measured using 2 different immunoassay platforms
    - Roche Diagnostics' Elecsys 2010 for CA125
    - Siemens Healthcare Diagnostics' BNII System for the others
  - Subsequently interpreted by the proprietary OvaCalc software

- **FDA-cleared blood test to help evaluate ovarian mass for cancer prior to a planned surgery.**

- Cut off premenopausal >5.0
  postmenopausal >4.4

FDA-cleared OVA1

– OVA1 has now been evaluated prospectively in a multi-institutional study examining 590 women scheduled for surgery for a pelvic mass

–

Performance	CA 125† (67 Units/mL) (n=524)	CA 125‡ (200 Units/mL) (n=524)	Multivariate Index Assay (n=524)	Physician Assessment (n=516)	Physician Assessment Plus Multivariate Index Assay (n=516)
Sensitivity (%)	77	69	93	75	96
n/N	124/161	111/161	149/161	121/161	154/161
95% CI	69.9–82.8	61.4–75.6	87.4–95.7	67.9–81.2	91.3–97.9
Specificity (%)	73	84	43	79	35
n/N	266/363	304/363	156/363	281/355	123/355
95% CI	77–87	79.6–87.2	38.0–48.1	74.6–83.1	29.9–39.7
PPV (%)	56	65	42	62	40
n/N	124/221	111/170	149/356	121/195	154/386
95% CI	49.5–62.5	57.9–72.0	36.8–47.0	55.1–68.6	35.1–44.9
NPV (%)	88	86	93	88	95
n/N	266/303	304/354	156/168	281/321	123/130
95% CI	83.6–91.0	81.9–89.1	87.9–95.9	83.5–90.7	89.3–97.4

Obstet Gynecol. 2011 Jun;117(6):1289-97

HE4 and Risk of Ovarian Malignancy Algorithm

- **HE4 is an additional new FDA-approved biomarker**
  - The FDA approved in 2008 for monitoring ovarian cancer
  - Recent development of a logistic regression model incorporating menopausal status with the serum levels of HE4 in combination with CA125 in the evaluation of women with a pelvic mass

- **The Risk of Ovarian Malignancy Algorithm (ROMA)**
  - Classifies into high-risk or low-risk groups for having epithelial ovarian cancer
  - The ROMA score reflects a predictive index calculated by equations that differ based on the patient's menopausal status.

TABLE 3 ROMA score calculation

Premenopausal women*

$PI = -12.0 + 2.38 \times LN[HE4] + 0.0626 \times LN[CA125]$

Postmenopausal women†

$PI = -8.09 + 1.04 \times LN[HE4] + 0.732 \times LN[CA125]$

Predicted probability

$\exp(PI)/[1 + \exp(PI)] \times 100$

Abbreviations: exp, exponential; LN, natural logarithm; PI, predictive index; ROMA, Risk of Ovarian Malignancy Algorithm.

*A score ≥12.5% is considered high risk in premenopausal women.

†A score ≥14.4% is considered high risk in postmenopausal women.

From Montagnana M, et al.[17]

Clin Chem Lab Med. 2011;49(3):521-525

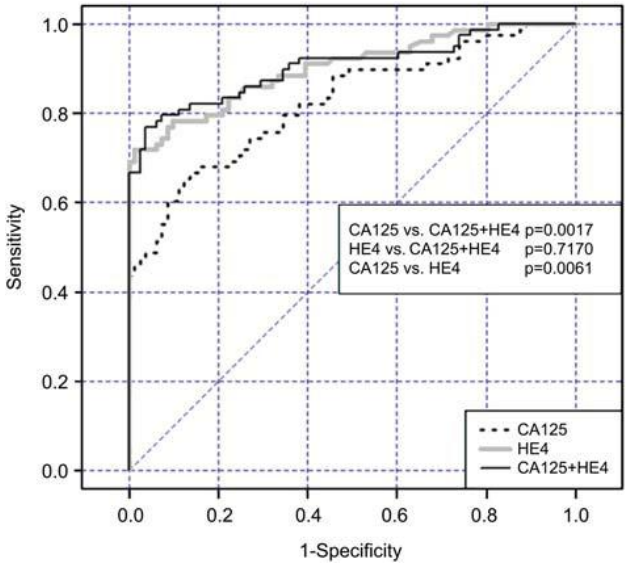
High Sensitivity with HE4/CA125 Combination

HE4/CA125

Tumor marker accuracy and **sensitivity at 90%, 95%, 98% specificity** for **ovarian cancer (n=78) vs. benign disease (n=81)**

Markers	p-Value for comparison of ROC-AUC to CA125	Sensitivity, %		
		At 90% specificity	At 95% specificity	At 98% specificity
All women				
CA125	–	60.3	51.3	46.2
HE4	0.0061	76.9	73.1	71.8
HE4+CA125	0.0017	79.5	76.9	69.2
Premenopausal				
CA125	–	59.5	48.7	43.2
HE4	0.1589	67.6	64.9	62.2
HE4+CA125	0.0600	73.0	70.3	67.6
Postmenopausal				
CA125	–	95.1	92.7	92.7
HE4	0.4351	85.4	80.5	80.5
HE4+CA125	0.4475	97.6	97.6	97.6

ROC curves for HE4, CA125, and CA125+HE4 for distinguishing **ovarian cancer (n=78) from a benign adnexal mass (n=81)**



Clin Chem Lab Med 2011; 49: 527-534

Prognosis (1)

- **Pre**operative serum CA-125 level
  - Value in localized/early-stage disease
    - Not in advanced ovarian cancer
  - 118 patients with FIGO stage I EOC
    - CA-125 <65 U/mL → longer survival
  - 600 patients with stage I
    - CA-125 ≤30 U/mL → good prognosis, possibly skip adjuvant tx.

- **Post**operative serum CA-125 level
  - CA125 of Post op 1month / CA125 of pre op

.

Prognosis (2)

- Serum CA-125 half-life during 1˚ chemotherapy
  - Independent prognostic factor in advanced EOC for complete remission and survival
  - m/c cut-off half-life: 20 days
- CA-125 level prior to 3rd chemotherapy, the slope of the CA-125 exponential regression curve, prechemotherapy CA125, nadir concentration, time to nadir
- Maintenance CTx
  - Initial baseline CA-125 ≤ 10 U/mL → superior progression free survival
- CA-125 level at relapse
  - Normal level (≤ 35U/mL) → better prognosis

Detecting Recurrence

- Serial CA-125 monitoring after initial chemotherapy
  - can detect recurrence early
  - Median lead-time: 63~99 days
- Santillan et al. study of 39 patients
  - 100% increased CA-125 → predict recurrence (OR: 23.7)
  - Associated with disease recurrence
    - ↑ 5 U/mL : OR=8.4; 95% CI 2.2-32.6
    - ↑ 10 U/mL : OR=71.2; 95% CI 4.8 ~ >999.9
- Patients in complete clinical remission
  - Progressive low-level increase in serum CA-125 → strongly predict disease recurrence

In Summary

Status of Current Tumor Markers in Ovarian Cancer_1

	Screening
Epithelial cancers	
Clinical practice	
Research	Serum CA-125 being assessed in screening trials in the general (UKCTOCS) and high-risk (UKFOCSS) and trials by GOG and CGN in the USA populations. Results from PLCO reported in 2012, results from the other trials expected in 2013/14. **Main emphasis on algorithms to interpret serial CA-125 and transvaginal ultrasound as a second-line test.**
Germ-cell tumors	
Clinical practice	
Research	
Sex cord stromal tumors	
Clinical practice	

Status of Current Tumor Markers in Ovarian Cancer_2

	Differential Diagnosis of an Adnexal Mass
Epithelial cancers	
Clinical practice	**CA-125 is the main marker-when combined with menopausal status and ultrasound features in the risk of malignancy index (RMI), a sensitivity of 71%-85% and specificity of 96%-97% is achieved.** CEA
Research	Inhibin pro-αC/total inhibin, kallikreins, mesothelin, prostasin, osteopontin, M-CSF, TPS, proteomic markers (profile, transthyretin, apolipoprotein A) CA-15-3, CA-72-4, CA-19-9, TATI, GAT, free serum DNA methylation, free glycans, IL-7, TNF α receptors HE4 microRNAs metabolite profiling
Germ-cell tumors	
Clinical practice	Serum AFP in tumors with endodermal sinus/yolk sac elements, serum β-hCG in tumors with chorionic elements
Research	M-CSF especially in dysgerminomas
Sex cord stromal tumors	
Clinical practice	Inhibib in granulosa-cell tumors

Status of Current Tumor Markers in Ovarian Cancer_3

	Prognositc Indicator
Epithelial cancers	
Clinical practice	**CA-125 levels after surgery and during chemotherapy are independent prognostic indicators. Various criteria are used based on CA-125 half-life.**
Research	Kallikrein 8, mesothelin, CYFRA 21-1, M-CSF, TATI, VEGF, CASA, tetranectin HE4
Germ-cell tumors	
Clinical practice	
Research	
Sex cord stromal tumors	
Clinical practice	

Status of Current Tumor Markers in Ovarian Cancer_4

	Monitoring Response to Therapy
Epithelial cancers	
Clinical practice	**Serial CA-125 levels reflects clinical course in 90% of positive tumors and are used routinely for monitoring patients.** HE4
Research	CASA
Germ-cell tumors	
Clinical practice	
Research	
Sex cord stromal tumors	
Clinical practice	Inhibin in granulosa cell tumors

Status of Current Tumor Markers in Ovarian Cancer_5

	Monitoring Disease and Recurrence
Epithelial cancers	
Clinical practice	**CA-125 detects recurrence with a sensitivity of 84%-94% and a false-positive rate of < 2%. Median lead-time compared with clinical diagnosis of recurrence is 60-99 days.** HE4
Research	Osteopontin, TPS CASA
Germ-cell tumors	
Clinical practice	
Research	
Sex cord stromal tumors	
Clinical practice	

In Conclusion

- Challenge still exists for the early diagnosis of ovarian cancer
- CA125 are useful marker for prognosis and disease monitoring.
- The use of multiple serum markers for the early diagnosis has not yet been established
- US FDA approved two algorithms, ROMA and OVA1 to estimate the risk of ovarian cancer in women with pelvic mass.

Thank you for your attention !