BME 301

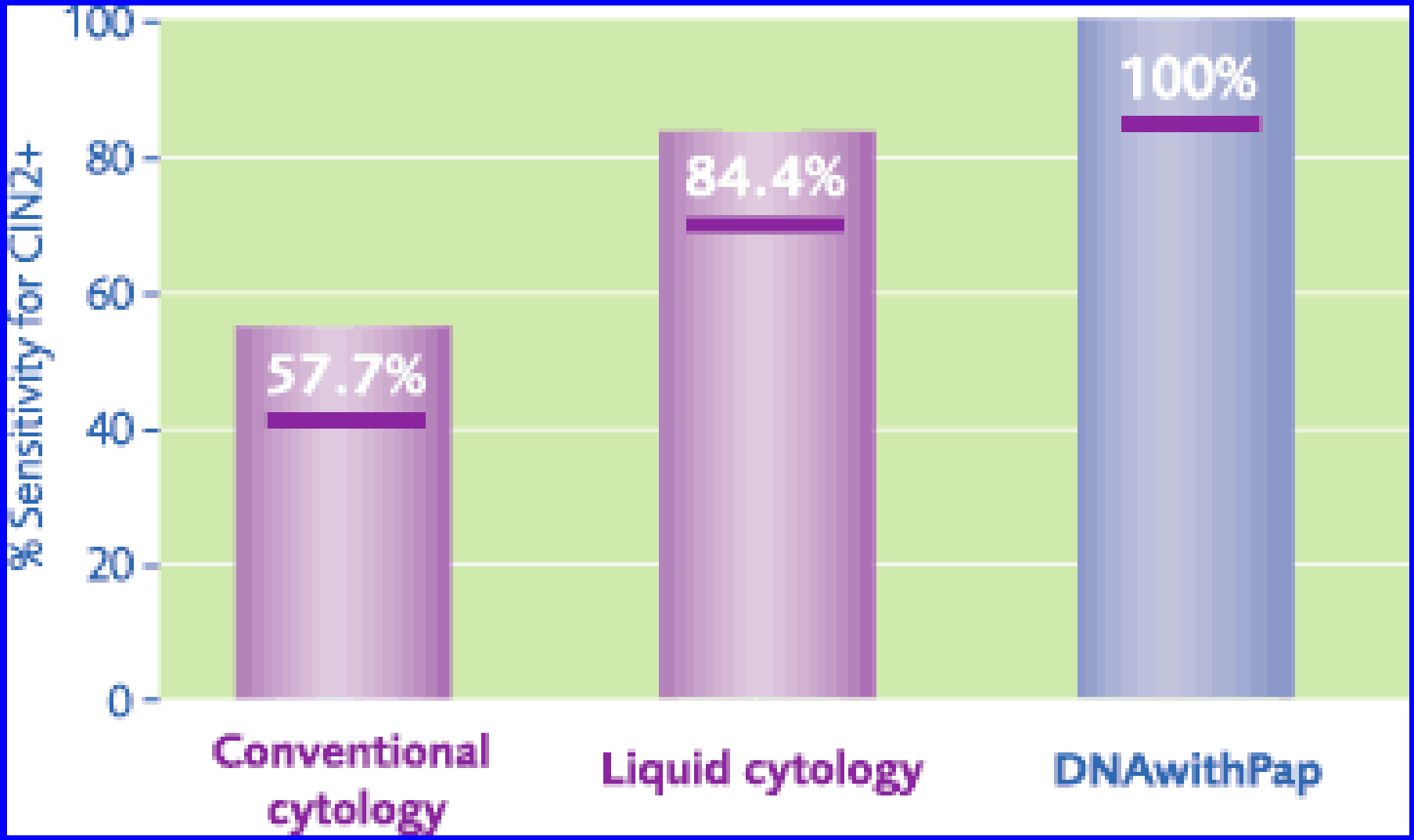
Lecture Twelve

HPV Testing

- The DNAwithPap Test is FDA-approved for routine adjunctive screening with a Pap test for women age 30 and older.
- Digene
  - http://www.digene.com

http://www.digene.com/PapXYLC-5301-30%20VER%20X.mpg

Sensitivity of HPV Testing



http://www.digene.com/images/sens.gif

Study of 5,671 women age >30 years

Comparison of Various Techniques

	Sensitivity	Specificity
Pap smear	60-80%	45-70%
Colposcopy	90-100%	20-50%
Digene HPV Test	80-90%	57-89%
VIA	67-79%	49-86%

Global Inequities in Cancer Prevention

HPV Vaccine

- 2006:
  - Gardasil vaccine to prevent HPV infection was licensed for use in girls & women ages 9-26 in USA and 48 other countries
  - Protects against 2 strains of HPV responsible for 70% of cervical cancers
- Non-infectious vaccine
  - Made by inserting gene for protein found in the HPV capsid into a different virus or yeast. Recombinantly produced HPV capsid protein self-assembles into virus like particles (VLPs).

HPV Vaccine Efficacy Trials

Manufacturer	Vaccine	Location	Participants	Projected End
Merck	VLPs of L1 protein from HPV 6/11/16/18, made in yeast, aluminum adjuvant	U.S., S. America, Europe	17,800 women, 16 to 26 years old	2007
		U.S., S. America, Europe, Asia	3800 women, 24 to 45 years old	2008
		U.S., S. America, Europe, Asia, Africa	3700 men, 16 to 24 years old	2008
GSK	VLPs of L1 protein from HPV 16/18, made in baculovirus, AS04 adjuvant	U.S., S. America, Europe, Asia Pacific	18,000 women, 15 to 25 years old	2010
		Costa Rica (run by NCI)	12,000 women, 18 to 25 years old	2010

HPV & Cervical Cancer

- Do condoms prevent HPV?
- Do we still need to screen women who have been vaccinated?

HPV Vaccine

- Gardasil
  - Protects against new HPV infections
  - Not effective for women who have already been exposed to HPV
- Gardasil:
  - Given as a series of 3shots over a 6 months
  - Cost: $360
  - This cost is a barrier even in developed countries, and is likely to limit its immediate impact in developing

HPV Vaccine

- HBV vaccine:
  - Licensed in 1981 in industrialized countries
  - 10 yrs before used in wealthier developing countries
  - >20 yrs before poorest countries had access
- Difficult to achieve widespread access to a vaccine targeted towards girls
  - Girls in developing countries less likely to be in school
  - Gender specific immunization may be culturally unacceptable

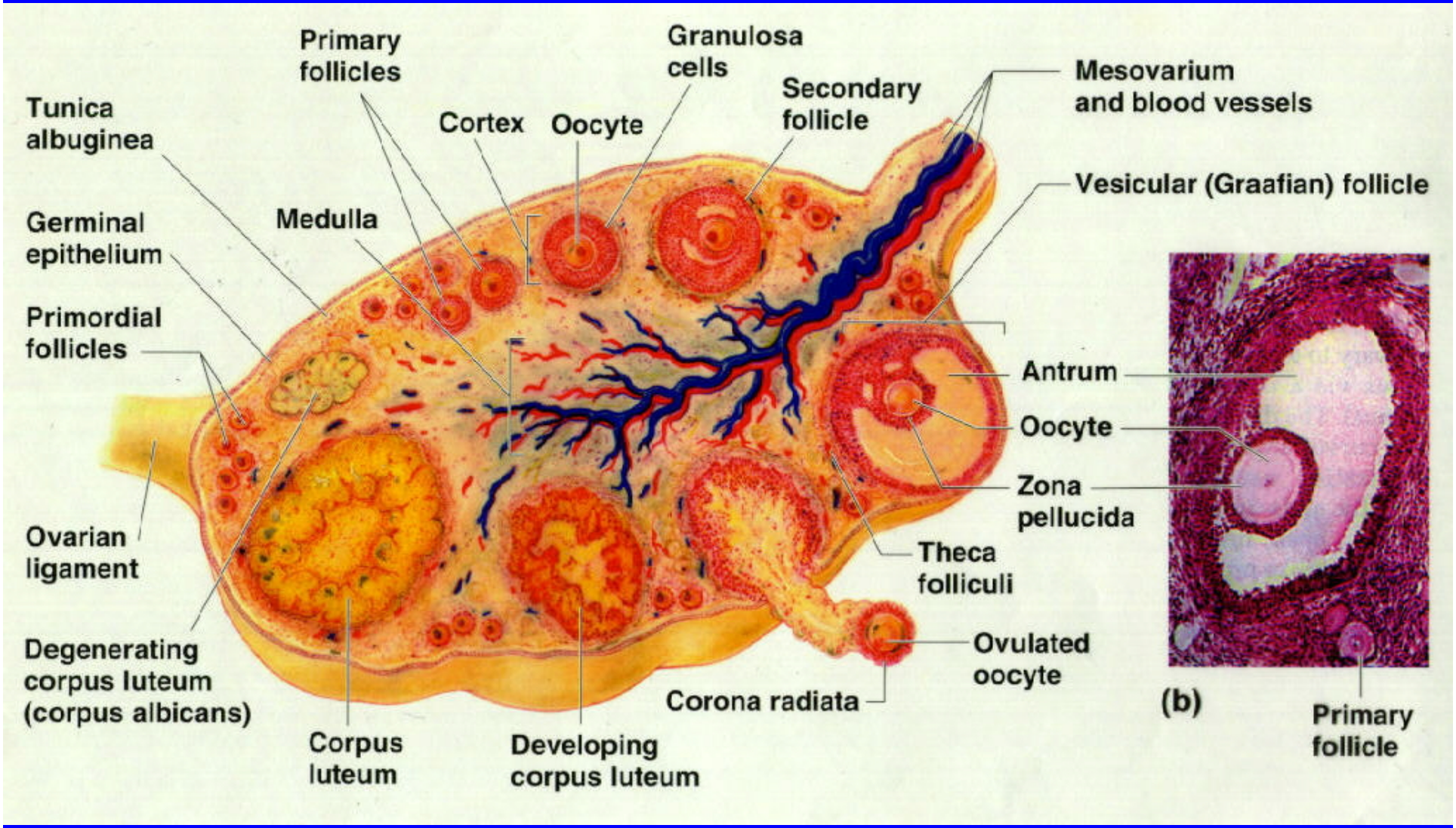
Summary of Cervical Cancer

- Cervical cancer
  - 2nd Leading cause of cancer death in women in world
  - Caused by infection with HPV
  - Precancer→cancer sequence
  - Precancer is very common
- Screening & Detection
  - Pap smear; colposcopy + biopsy
  - Reduces incidence and mortality of cervical cancer
  - Insufficient resources to screen in developing countries
- New technologies
  - Automated reading of Pap smears → reduce FN rate
  - HPV testing
  - VIA

Ovarian Cancer

Early Detection

Ovary



http://www.vacadsci.org/jsr/ovary.jpg

Ovarian Cancer

- Screening:
  - No adequate screening tests available
- Treatment:
  - Surgery, radiation therapy, chemotherapy
- 5 year survival
  - All stages: 53%
  - Localized disease (Stage I): 90%
  - Metastatic (Stage III-IV): 15-20%
    - 70% of women diagnosed at these stages

Ovarian Cancer “Whispers”

- Symptoms:
  - Unexplained change in bowel and/or bladder habits such as constipation urinary frequency, incontinence
  - Gastrointestinal upset: gas, indigestion, nausea
  - Unexplained weight loss or weight gain
  - Pelvic and/or abdominal pain or discomfort
  - Pelvic and/or abdominal bloating or swelling
  - A constant feeling of fullness
  - Fatigue
  - Abnormal or postmenopausal bleeding
  - Pain during intercourse

Early Detection of Ovarian Cancer

- Pelvic and rectal examination:
  - Feel uterus and ovaries to find abnormality in shape or size
  - Unlikely to detect early stage ovarian cancer
- CA-125:
  - 80% of women with advanced ovarian cancer have elevated CA125
  - Used to monitor ovarian cancer after diagnosis is surgically confirmed - sensitive indicator of persistent or recurrent disease
  - Very unreliable for detecting early cancer
  - Very unreliable for detecting cancer in pre-menopausal women
    - Elevated by conditions such as pregnancy, endometriosis, uterine fibroids, liver disease, and benign ovarian cysts
- Transvaginal Ultrasound:
  - Use high-frequency sound waves to create pictures of ovaries
  - Can detect ovarian malignancies in asymptomatic women
  - Poor accuracy in detecting early stage disease

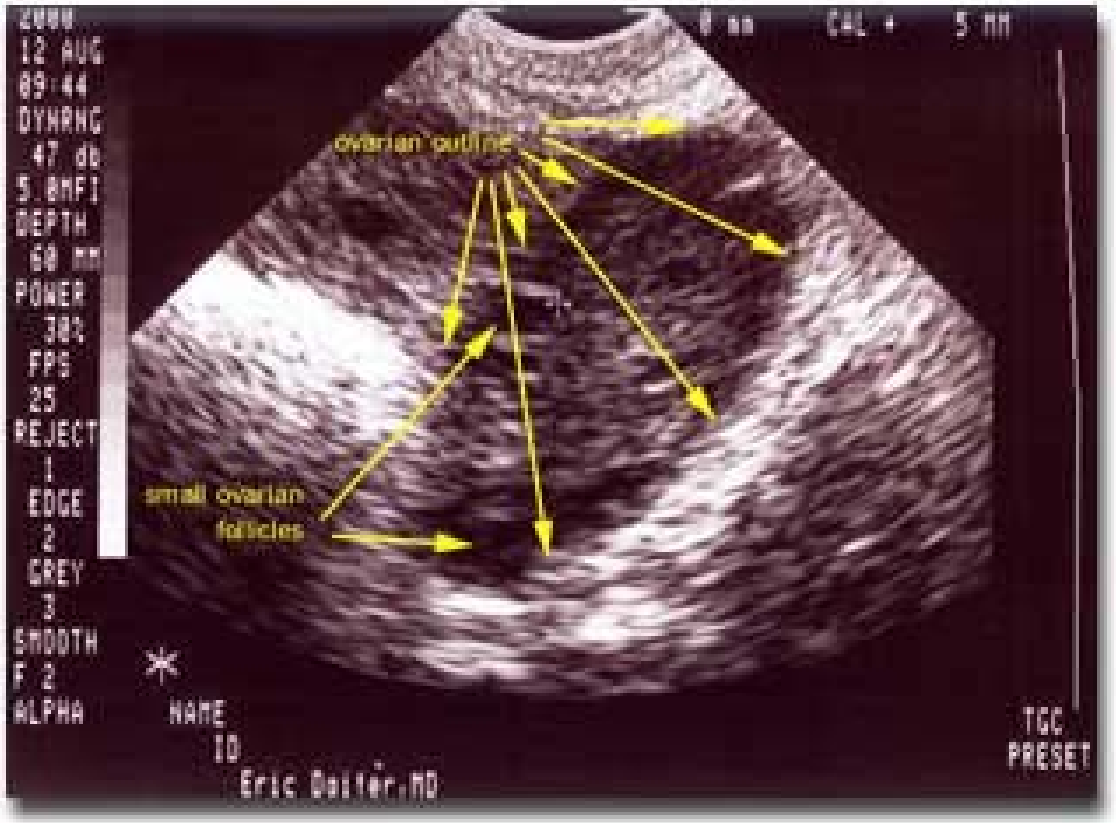
Performance of CA125

- Overall performance in Norwegian study:
  - Sensitivity of 30-35%
  - Specificity was 95.4%
- Performance by stage:
  - Sensitivity for Stage I cancers: 29 - 75%
  - Sensitivity for Stage II cancers: 67 - 100%

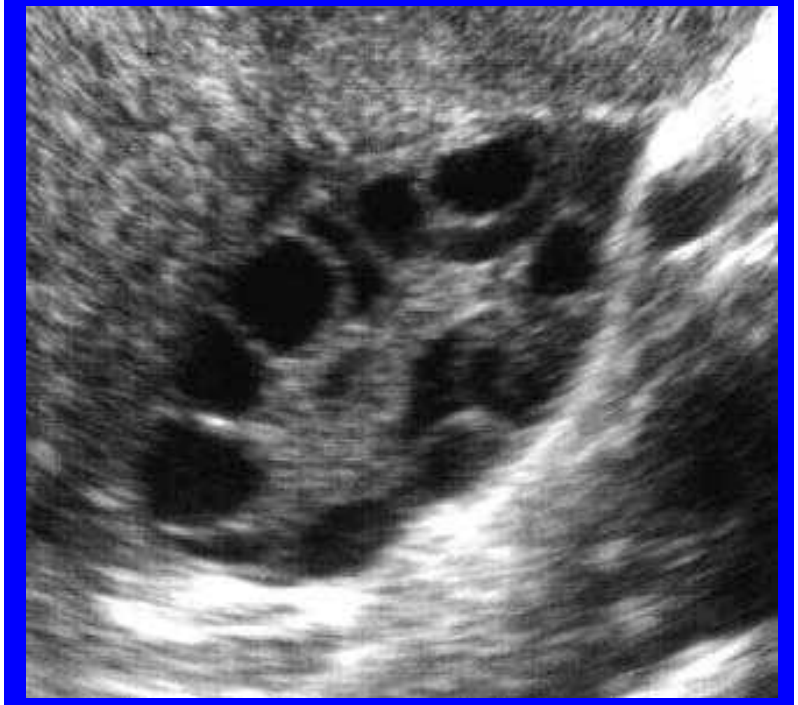
Transvaginal Sonography

- Sensitivity = 100%
- Specificity = 96%

http://www.infertilitytutorials.com/images/transvaginal_ultrasound.jpg

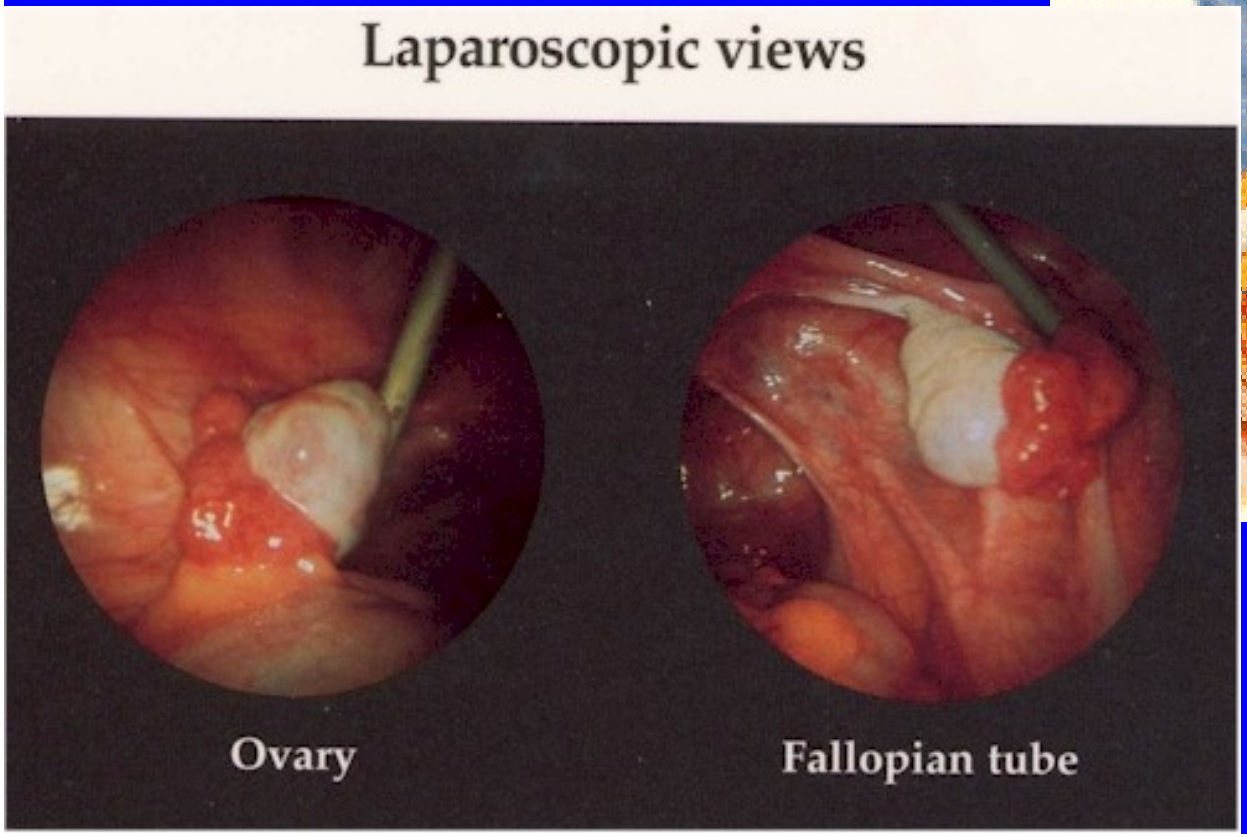


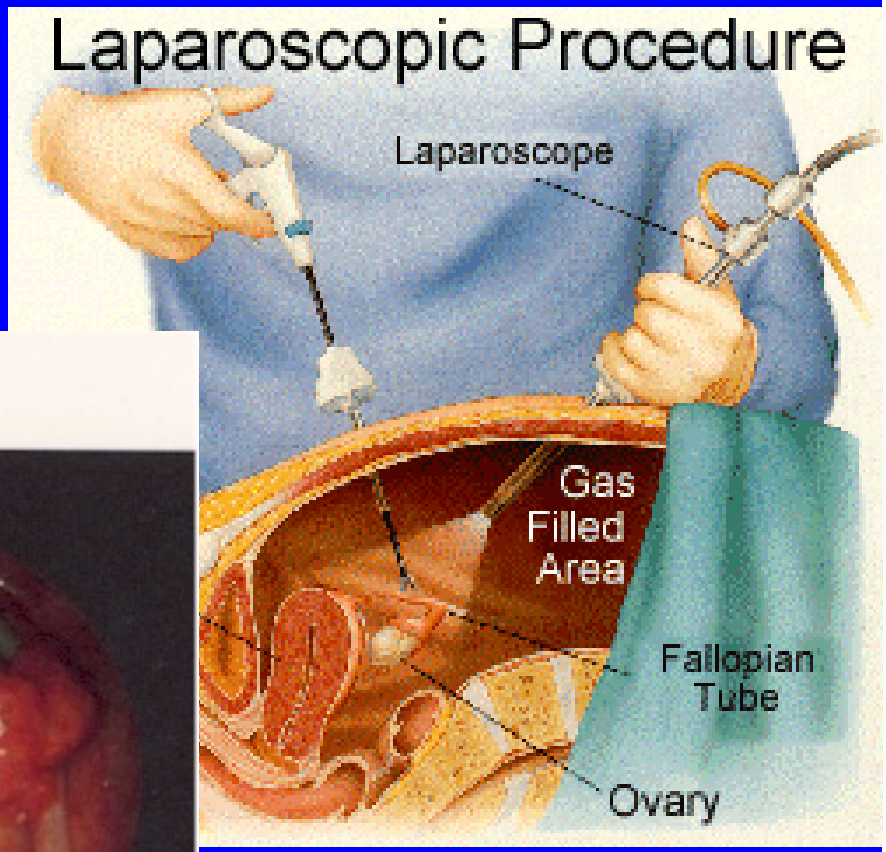
http://www.ivf-infertility.com/images/polycystic_ovary.jpg



Diagnostic Laparoscopy

Complication Rate =
0.5 – 1%





http://www.aiof.com/html/images/lapro.jpg

Screening Scenarios

- Scenario #1:
  - Screen 1,000,000 women with CA125
    - p = .0001 (100 cancers)
    - Se=35%, Sp=98.5%
    - Cost = $30
  - Follow with laparoscopy
    - Complication rate = 1%
    - Cost=$2,000
- TP=35 FP=14,999 Complications=150
- PPV =0.23% NPV =99.99%
- Cost per cancer found = $1,716,200

Screening Scenarios

- Scenario #2:
  - Screen 1,000,000 women with transvaginal US
    - P = .0001 (100 cancers)
    - Se=100%, Sp=96%
    - Cost = $150
  - Follow with laparoscopy
    - Complication rate = 1%
    - Cost=$2,000
- TP=100 FP=39,996 Complications=401
- PPV =0.25% NPV =100%
- Cost per cancer found = $300,672

Screening Scenarios

- Scenario #3:
  - Screen 1,000,000 women >age 50 with TVUS
    - P = .0005 (500 cancers)
    - Se=100%, Sp=96%
    - Cost = $150
  - Follow with laparoscopy
    - Complication rate = 1%
    - Cost=$2,000
- TP=500 FP=39,980 Complications=405
- PPV =1.24% NPV =100%
- Cost per cancer found = $60,670

Screening Scenarios

- Scenario #3 cont.:
  - Screen 1,000,000 women > age 50 with TVUS
    - P = .0005 (500 cancers)
    - Se=100%, Sp=??%
    - Cost = $150
  - How high does Sp need to be for PPV to reach 25%?
    - Sp = 99.985%

Does Ultrasound Screening Work?

- Two studies of over 10,000 low-risk women:
  - The positive predictive value was only 2.6%
  - Ultrasound screening of 100,000 women over age 45 would:
    - Detect 40 cases of ovarian cancer,
    - Result in 5,398 false positives
    - Result in over 160 complications from diagnostic laparoscopy
  - Jacobs I. Screening for early ovarian cancer. Lancet; 2:171-172, 1988.

Ongoing Trials

- United Kingdom
  - 200,000 postmenopausal women
    - CA 125 level plus transvaginal ultrasound examination
    - Transvaginal ultrasound alone
    - No screening
- United States:
  - 37,000 women (aged 55–74)
    - Annual CA 125 level and transvaginal ultrasound examination
    - No screening
- Europe:
  - 120,000 postmenopausal women
    - No screening,
    - Transvaginal ultrasound at intervals of 18 months
    - Transvaginal ultrasound at intervals of 3 years

http://www.mja.com.au/public/issues/178_12_160603/and10666_fm.pdf

New Screening Tool

- Current screening tests look for 1 protein:
  - CA125
  - PSA
- Many serum proteins
- Can complex fingerprint predictive of cancer can be identified?
- PROTEOMICS:
  - Don’t try to understand disease mechanisms
  - Use proteomics to analyze patterns made by all proteins in the blood, without even knowing what they are

In The News

http://msnbc.msn.com/id/3933580/

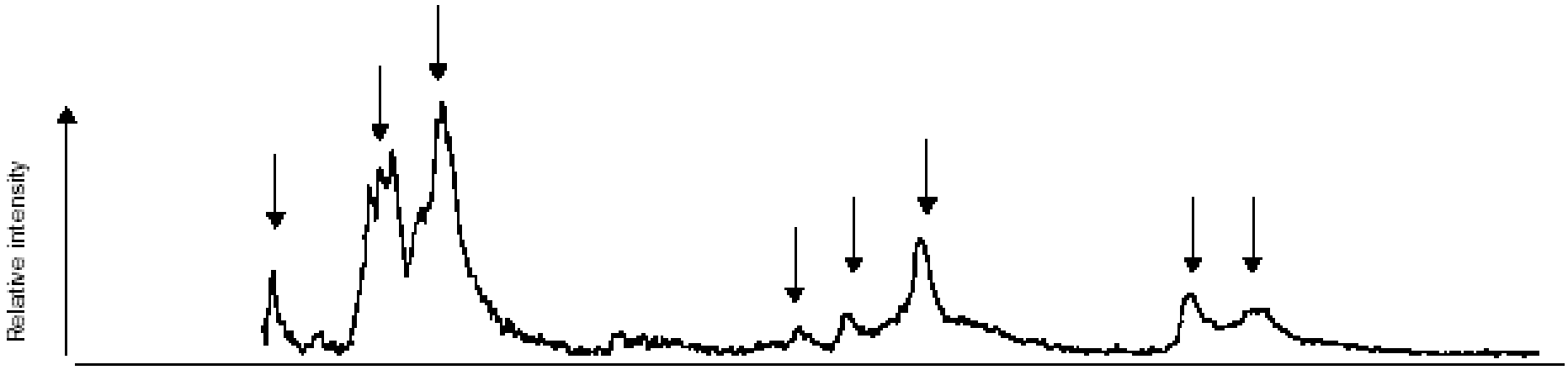
New Screening Tool

- Blood test to detect ovarian cancer
  - Examined thousands of proteins
  - Found a few that appear to be hallmarks of ovarian cancer
  - Se = 50/50 = 100%
  - Sp = 63/66 = 95%
  - PPV = 94%
- "The most important next goal is validating the promise of these results in large, multi-institutional trials."
  - Lance Liotta, M.D., Ph.D.

How do we measure serum proteins?

- Mass Spectrometry:
  - Serum proteins are vaporized, given an electric charge and propelled down a tube
  - How fast they make the trip depends on their mass
  - Produces graph that shows distribution of masses in the sample
  - Use computer program to analyze patterns and distinguish blood from patients with cancer and from those without

Typical Data



mass/charge

15,200 values of intensity vs. mass/charge

Mechanisms of disease

Use of proteomic patterns in serum to identify ovarian cancer

Emanuel F Petricoin III, Ali M Ardekani, Ben A Hitt, Peter J Levine, Vincent A Fusaro, Seth M Steinberg, Gordon B Mills, Charles Simone, David A Fishman, Elise C Kohn, Lance A Liotta

Summary

Background New technologies for the detection of early-stage ovarian cancer are urgently needed. Pathological changes within an organ might be reflected in proteomic patterns in serum. We developed a bioinformatics tool and used it to identify proteomic patterns in serum that distinguish neoplastic from non-neoplastic disease within the ovary.

Methods Proteomic spectra were generated by mass spectroscopy (surface-enhanced laser desorption and ionisation). A preliminary "training" set of spectra derived from analysis of serum from 50 unaffected women and 50 patients with ovarian cancer were analysed by an iterative searching algorithm that identified a proteomic pattern that completely discriminated cancer from non-cancer. The discovered pattern was then used to classify an independent set of 116 masked serum samples: 50 from women with ovarian cancer, and 66 from unaffected women or those with non-malignant disorders.

Findings The algorithm identified a cluster pattern that, in the training set, completely segregated cancer from non-cancer. The discriminatory pattern correctly identified all 50 ovarian cancer cases in the masked set, including all 18 stage I cases. Of the 66 cases of non-malignant disease, 63 were recognised as not cancer. This result yielded a sensitivity of 100% (95% CI 93–100), specificity of 95% (87–99), and positive predictive value of 94% (84–99).

Interpretation These findings justify a prospective population-based assessment of proteomic pattern technology as a screening tool for all stages of ovarian cancer in high-risk and general populations.

Lancet 2002; **359:** 572–77

Introduction

Application of new technologies for detection of ovarian cancer could have an important effect on public health,[1] but to achieve this goal, specific and sensitive molecular markers are essential.[1–3] This need is especially urgent in women who have a high risk of ovarian cancer due to family or personal history of cancer, and for women with a genetic predisposition to cancer due to abnormalities in predisposition genes such as *BRCA1* and *BRCA2*. There are no effective screening options for this population.

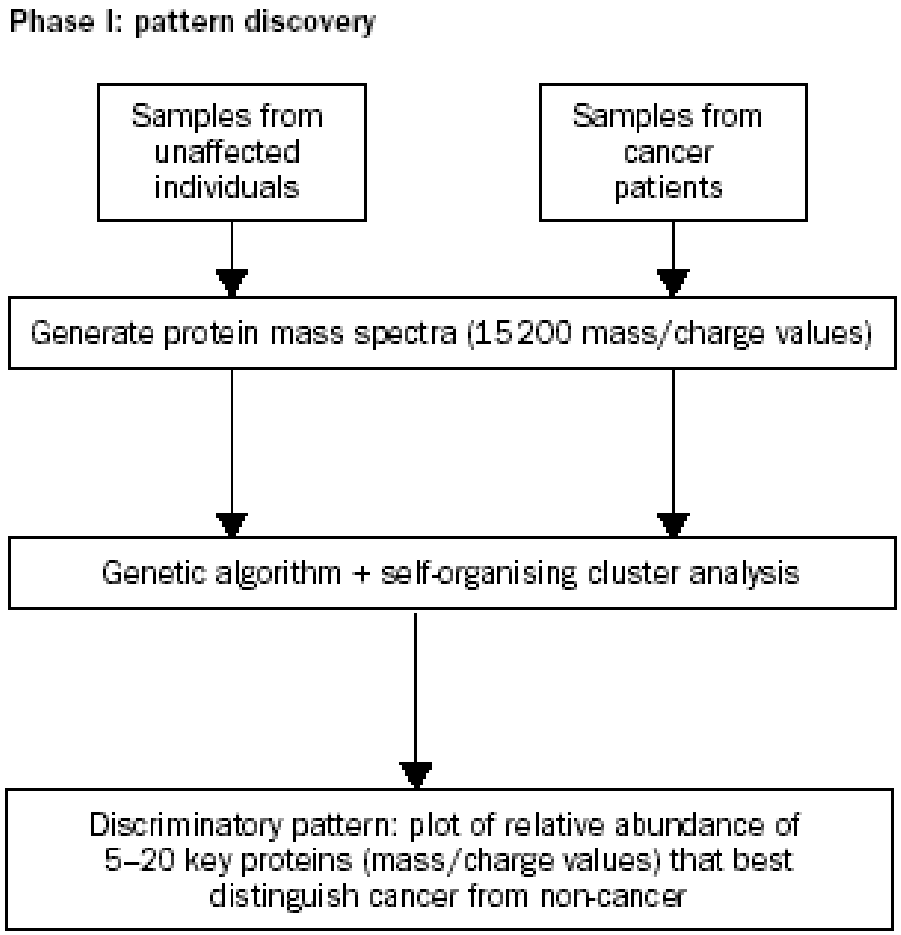
Ovarian cancer presents at a late clinical stage in more than 80% of patients,[1] and is associated with a 5-year survival of 35% in this population. By contrast, the 5-year survival for patients with stage I ovarian cancer exceeds 90%, and most patients are cured of their disease by surgery alone.[1–6] Therefore, increasing the number of women diagnosed with stage I disease should have a direct effect on the mortality and economics of this cancer without the need to change surgical or chemotherapeutic approaches.

Cancer antigen 125 (CA125) is the most widely used biomarker for ovarian cancer.[1–6] Although concentrations of CA125 are abnormal in about 80% of patients with advanced stage disease, they are increased in only 50–60% of patients with stage I ovarian cancer.[1–6] CA125 has a positive predictive value of less than 10% as a single marker, but the addition of ultrasound screening to CA125 measurement has improved the positive predictive value to about 20%.[6]

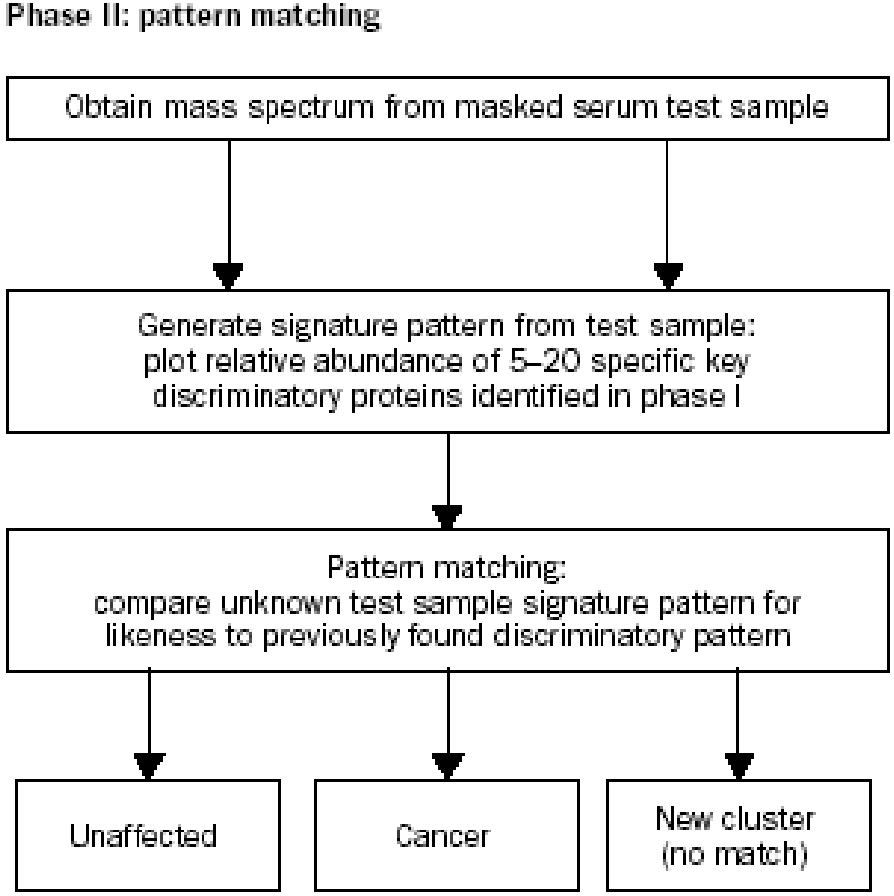
Low-molecular-weight serum protein profiling might reflect the pathological state of organs and aid in the early detection of cancer. Matrix-assisted laser desorption and ionisation time-of-flight (MALDI-TOF) and surface-enhanced laser desorption and ionisation time-of-flight (SELDI-TOF) mass spectroscopy can profile proteins in this range.[6–9] These profiles can contain

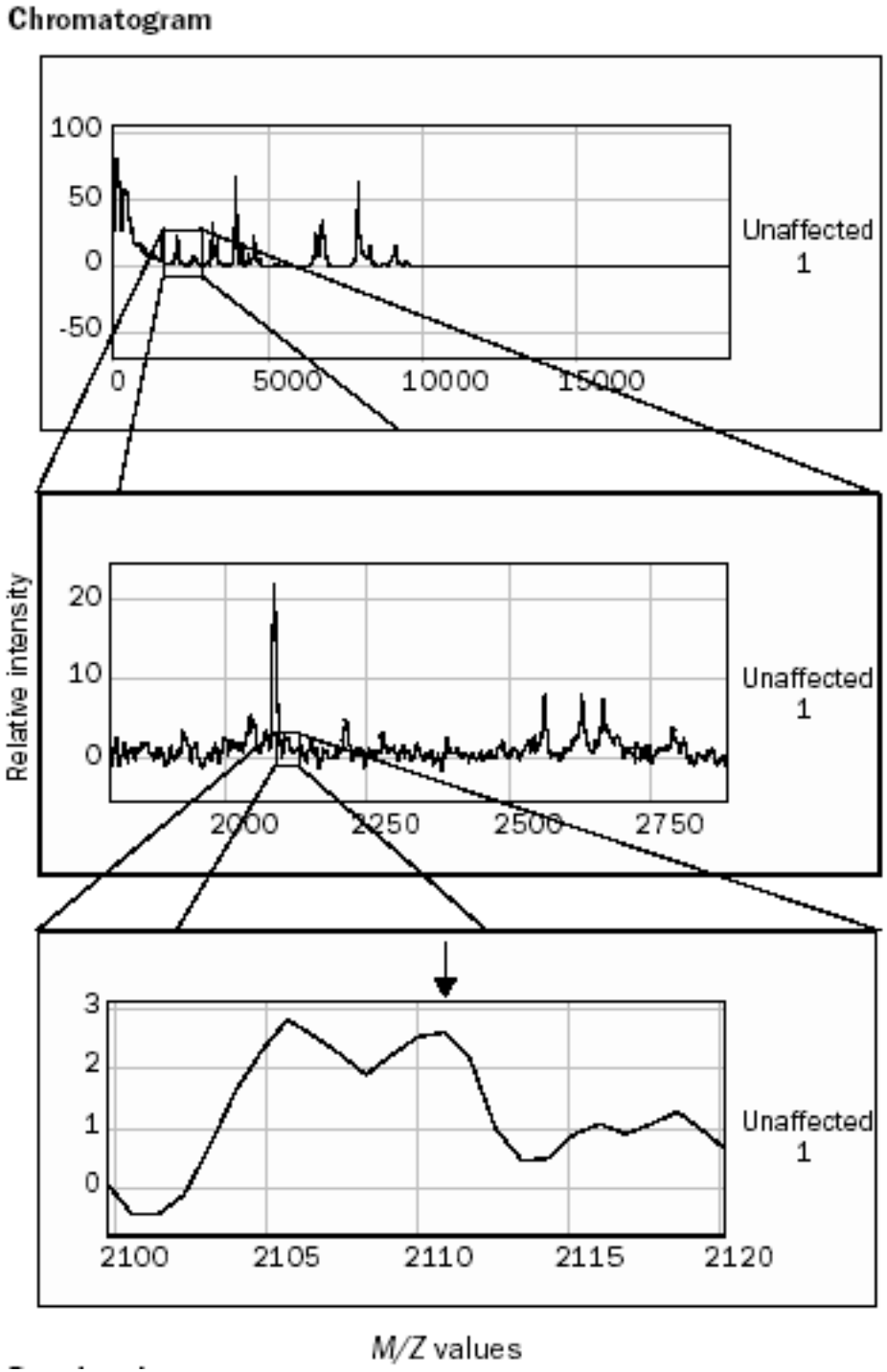
Data Analysis

Training



Validation





Useful M/Z:

534

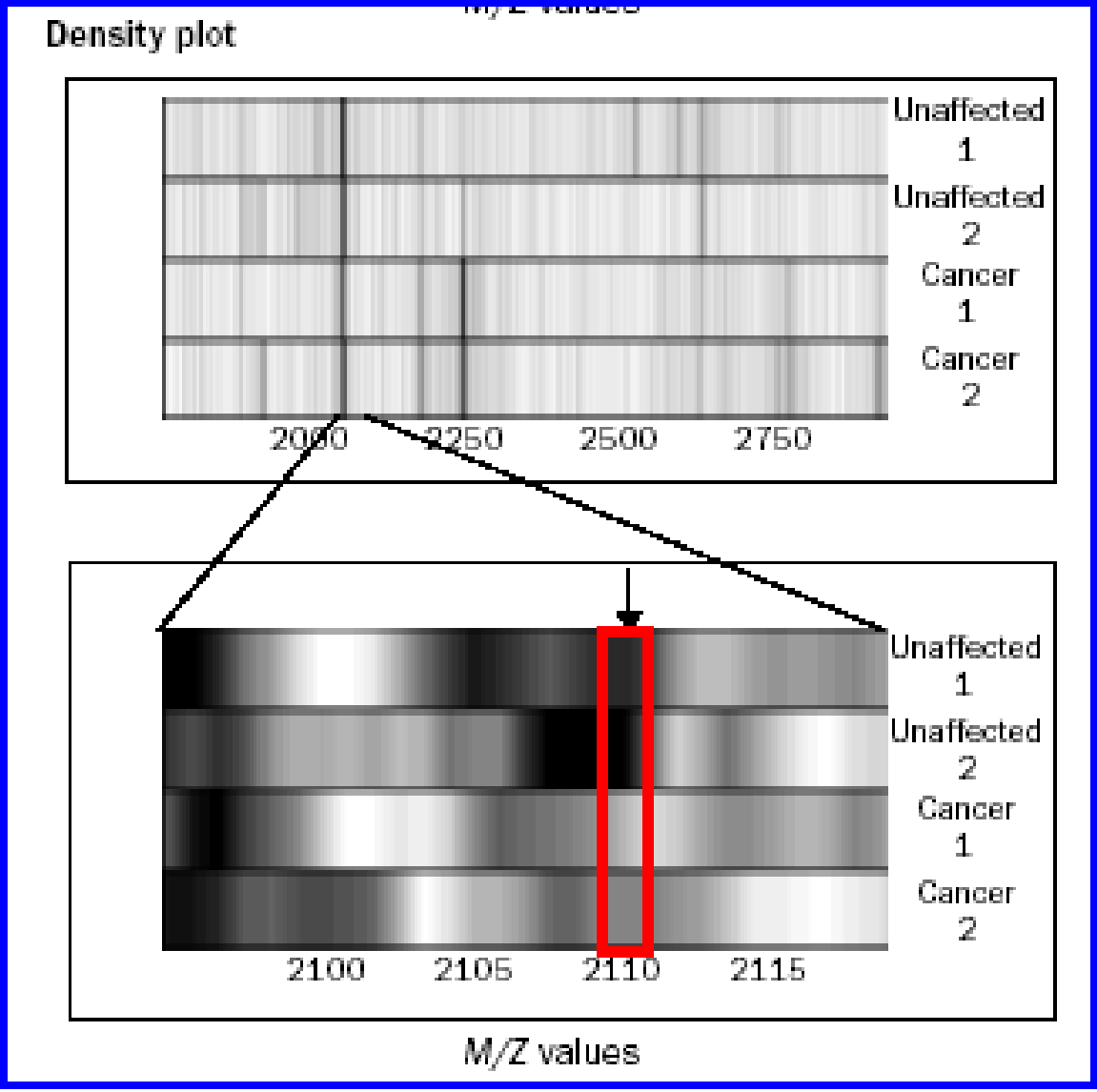
989

2111

2251

2465

Comparative Analysis



Useful M/Z:

534

989

2111

2251

2465

OvaCheck

- Quest Diagnostics and LabCorp:
  - Will analyze blood samples sent by doctors, rather than sell test kits to doctors and hospitals
  - Tests performed at a central location do not require F.D.A. approval
  - Will be available in a few months
  - Cost: $100-$200

Response

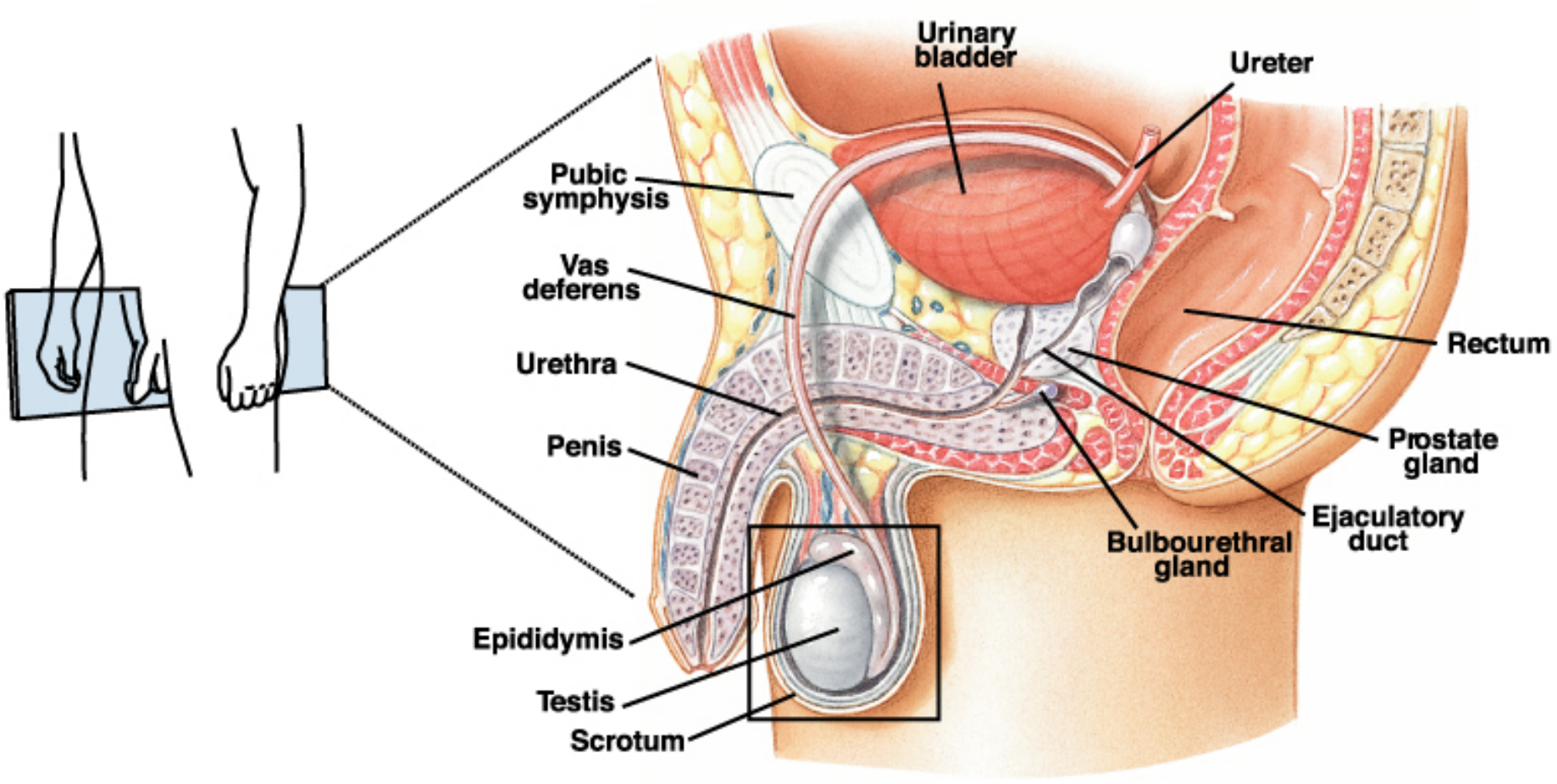
- Dr. Eleftherios P. Diamandis, head of clinical biochem at Mount Sinai Hospital in Toronto.
  - "If you don't know what you're measuring, it's a dangerous black-box technology... They are rushing into something and it could be a disaster."
- Dr. Nicole Urban, head of gynecologic cancer research at the Fred Hutchinson Cancer Research Center in Seattle.
  - "Certainly there's no published work that would make me tell a woman she should get this test."
- Dr. Beth Karlan, director of gynecologic oncology at Cedars-Sinai Medical Center
  - "Before you mass-market to the uninformed, fearful population, it should be peer-reviewed,"
  - When asked whether she would recommend her patients not get tested, she said: "It doesn't matter what I recommend. They are going to do it anyway."

http://www.ovarian.org/press.asp?releaseID=263

Prostate Cancer

Early Detection

http://cwx.prenhall.com/bookbind/pubbooks/silverthorn2/medialib/Image_Bank/CH24/FG24_09a.jpg

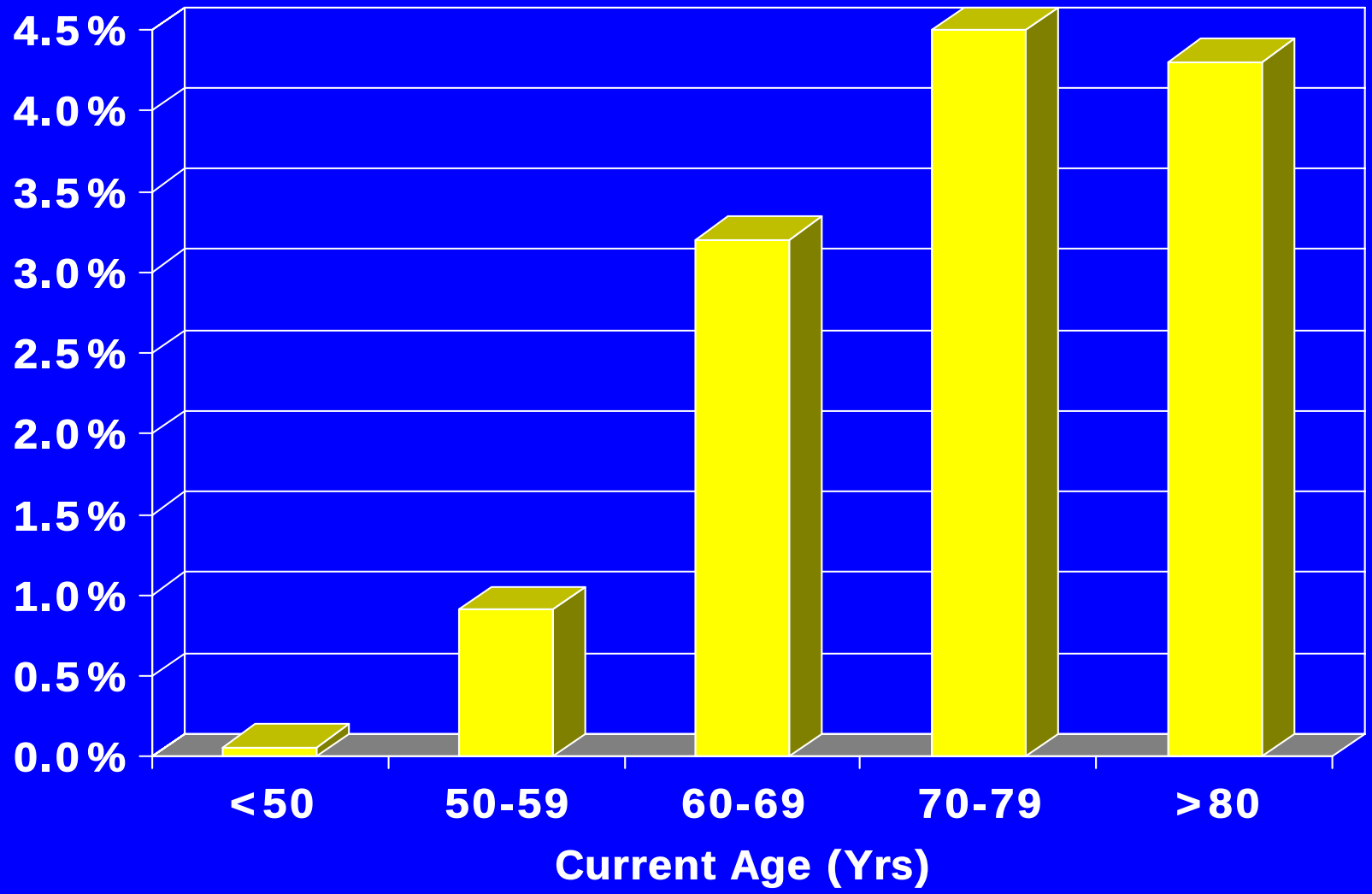


Prostate gland contributes enzymes, nutrients and other secretions to semen.

Prostate Cancer: Statistics

- United States:
  - 230,110 new cases in US
  - 29,900 deaths in US
  - 2nd leading cause of cancer death in men
- Worldwide:
  - 543,000 new cases each year
  - Third most common cancer in men
- Risk Factors:
  - Age
  - Race (incidence 3X higher in African Americans)
  - Family history of prostate cancer

Risk of Prostate Cancer in Next 5 Yrs



Development of Prostate Cancer

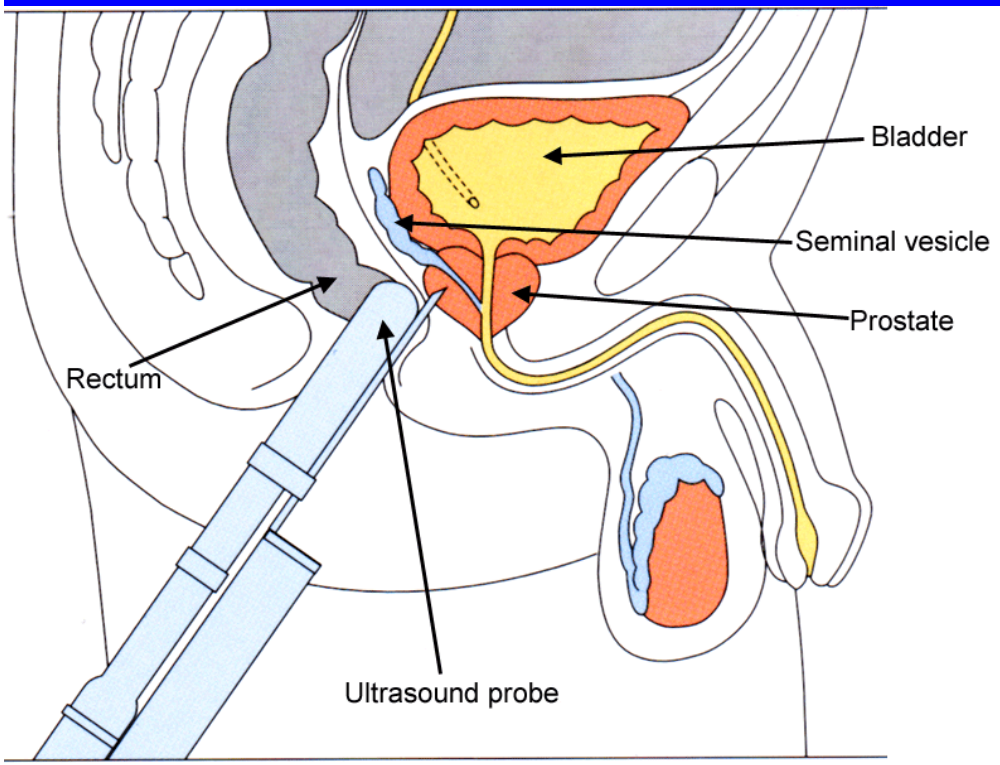
- Prostate Cancer:
  - Slow, but continuously growing neoplasia
  - Preclinical form develops at age 30
  - Remains latent for up to 20 years
  - Can progress to aggressive, malignant cancer
- Peak incidence: 7th decade of life
- Signs and symptoms:
  - Often asymptomatic in early stages
  - Weak or interrupted urine flow
  - Inability to urinate
  - These are symptoms of prostate enlargement

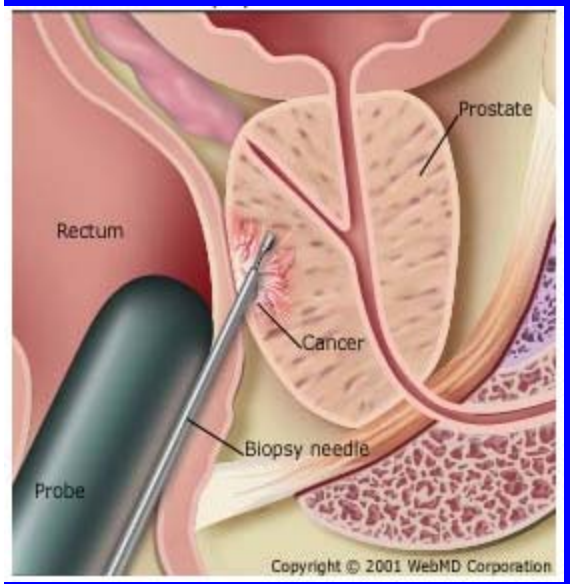
Prostate Cancer (2005)

- Screening (American Cancer Society recs):
  - Annual serum PSA test beginning at age 50
  - Annual digital rectal exam at age 50
- Treatment:
  - Surgery, radiation therapy, hormone therapy, chemotherapy
- 5 year survival
  - All stages: 98%
  - Localized disease: 100%
  - Distant metastases: 31%

What happens if DRE & PSA are +?

- Biopsy of prostate ($1500)
  - Insert needle through wall of rectum into prostate
  - Remove fragments of prostate
  - Examine under microscope





http://my.webmd.com/NR/rdonlyres/0557C509-969D-4441-A7BE-1236F9623C2F.jpeg

Rx for Localized Prostate Cancer

- Radical prostatectomy (remove prostate)
  - Usually curative
  - Serious side effects:
    - Incontinence (2-30%)
    - Impotence (30-90%)
    - Infertility
- Conservative management
  - Just watch until symptoms develop

Does Early Detection Make a Δ?

- 10 Yr Survival Rates for Localized Prostate CA:
  - Grade I:
    - Surgery 94%
    - Conservative Rx 93%
  - Grade II:
    - Surgery 87%
    - Conservative Rx 77%
  - Grade III:
    - Surgery 67%
    - Conservative Rx 45%
- Makes a difference only for high grade disease

Challenges of Screening

- Prostate cancer is a slow-growing cancer
  - Not symptomatic for an average of 10 years
- Most men with prostate cancer die of other causes
- Treatment has significant side effects
- 50 year old man:
  - 40% chance of developing microscopic prostate cancer
  - 10% chance of having this cancer diagnosed
  - 3% chance of dying of it

Should we screen?

- Yes:
  - Localized prostate cancer is curable
  - Advanced prostate cancer is fatal
  - Some studies (not RCTs) show decreased mortality in screened patients
- No:
  - False-positives lead to unnecessary biopsies
  - Over-detection of latent cancers
    - We will detect many cancers that may never have produced symptoms before patients died of other causes (slow growing cancer of old age)
  - No RCTs showing decreased mortality

Clinical Evidence

- Three case-control studies of DRE
  - Mixed results
- One completed RCT of DRE & PSA
  - Found no difference in # of prostate cancer deaths between groups randomized to screening and usual care

Randomized Clinical Trials Underway

- Prostate Cancer vs. Intervention Trial (US)
- Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (US)
- European Randomized Study for Screening for Prostate Cancer
  - 239,000 men
  - 10 countries
  - Will be complete in 2008

Do All Countries Screen with PSA?

- United States:
  - Conflicting recommendations
- Europe:
  - No
  - Not enough evidence that screening reduces mortality

Conflicting Recommendations in US

- Guide to Clinical Preventive Services
  - Do NOT screen using DRE or serum PSA
- American College of Preventive Medicine
  - Men aged 50 or older with >10 yr life expectance should be informed and make their own decision
- American Cancer Society (and others)
  - Men aged 50 or older with > 10 yr life expectancy should be screened with DRE and serum PSA

PSA Test

Details

The PSA Test

- What is PSA?
  - Prostate-specific antigen
  - A glycoprotein responsible for liquefaction of semen
  - Highly specific for prostate (only made by the prostate)
- PSA test is a blood test to measure PSA levels
- Why measure PSA to screen for cancer?
  - PSA levels are closely (but not definitively) associated with prostate cancer
    - May be elevated in benign conditions (BPH, Prostatitis)
    - Not always high in cancer
- Cost:
  - $30-$100

PSA Levels

- Normal PSA Levels:
  - < 4 ng/ml
  - Can vary by age
    - 40-49 yo < 2.5 ng/ml
    - 50-59 yo < 3.5 ng/ml
    - 60-69 yo < 4.5 ng/ml
    - 70-80 yo < 6.5 ng/ml
- Cancer Patients:
  - 20-25% have PSA < 4 ng/ml
  - 20-25% have 4 ng/ml < PSA < 10 ng/ml
  - 50-60% have PSA > 10 ng/ml

Sensitivity and Specificity of PSA

- How to determine
  - Trial: Serum PSA → Biopsy (Gold standard)
    - If BX is positive and PSA is positive: get TP
    - If BX is positive and PSA is negative: get FN
    - If BX is negative and PSA is negative: get TN
    - If BX is negative and PSA is positive: get FP
    - BUT: if BX is negative:
      - Did BX just fail to sample area with cancer?
  - Hard to calculate Specificity - TN/(TN+FP)
- Cutpoint of 4 ng/ml
- Sensitivity = 63-83%
- Specificity = 90%

Predictive Value Calculation

- Screening Performance:
  - Se = 73%; Sp = 90%
- Number Tested:
  - N=1,000,000; Prevalence = 2%
- Costs:
  - Screening = $30; Follow up biopsy = $1500
- What are PPV & NPV?
- What is screening cost?
- What is biopsy cost?
- What is cost/cancer found?

PSA Example – Predictive Value

	Test Positive	Test Negative	
Disease Present	14,600	5,400	# with Disease = 20,000
Disease Absent	98,000	882,000	#without Disease = 980,000
	# Test Pos = 112,600	# Test Neg = 887,400	Total Tested = 1,000,000

PPV =14,600/112,600 = 13%

NPV =882,000/887,400 = 99%

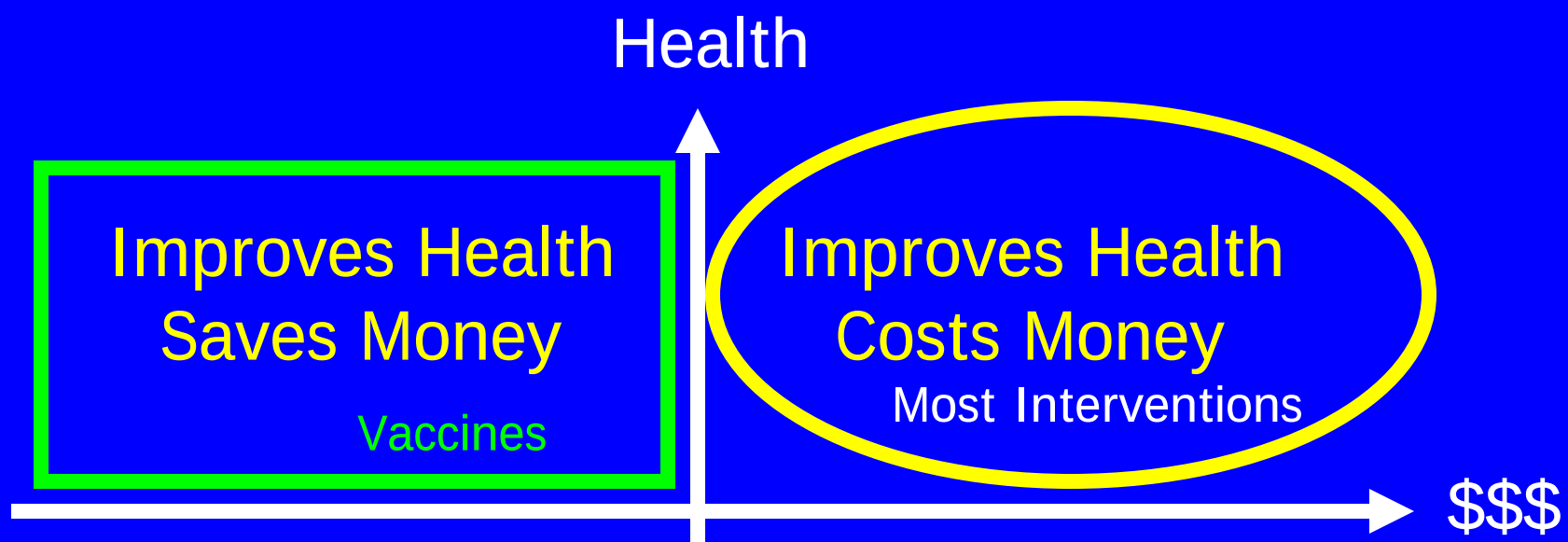
PSA Example – Cost

	Test Positive	Test Negative	
Disease Present	14,600	5,400	# with Disease = 20,000
Disease Absent	98,000	882,000	#without Disease = 980,000
	# Test Pos = 112,600	# Test Neg = 887,400	Total Tested = 1,000,000

Cost to Screen =$30*1,000,000+$1500*112,600 =$168,900,000

Cost/Cancer = $168,900,000/14,600=$13,623

Health – Policy Space



Worsens Health
Saves Money

??????????????????

Worsens Health
Costs Money

http://www.npr.org/templates/story/story.php?storyId=93313794

New Technologies: Improved Screening

- Additional serum markers→ Improve Sp
  - Free PSA
  - PSA density
  - PSA velocity
- Predict those cancers which will progress to advanced disease
  - Gene chips

Cancer Screening Exams

- Cellular Changes
  - Pap smear
- Serum Proteins
  - PSA
  - CA125
  - OvaCheck
- Genetic Changes
  - HPV DNA
  - Mutations in the BRCA1, BRCA2 genes → Risk