The Detection of Early Stage Epithelial Ovarian Cancer

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The problem:

Epithelial Ovarian Carcinoma

• 70-75% women are diagnosed with advanced disease (as in 1960)
• Poor 5-year survival (12-15%) for advanced stage EOC
• 90% 5-year survival for stage I disease—yet often detected serendipitously
• Therefore intentional detection of early stage disease is critical

Estimated Gynecologic Cancer Deaths 2007

- Ovary, 15,280 (54%)
- Uterine, 6,350 (22%)
- Cervix, 2,780 (18%)
- Other, 2,000 (6%)

27,100 (10%)
The Cause:
Epithelial Ovarian Cancer

• Serous (45%)
• Mucinous (13%)
• Endometrioid (15%)
• Clear Cell (3%)
• Brenner (2%)
• Mixed Carcinoma
• Undifferentiated Carcinoma

How to Detect Early Stage EOC???

• Annual CA125 and US do not achieve detection of early stage disease
• Both can provide false security or inappropriate anxiety
• Accuracy approximates 50% for early stage disease

CA125

• increased in 80% postmenopausal women with OVCA, yet at best 50% with Stage I disease.
• many causes of false elevations; fibroids, benign ovarian cysts, pelvic inflammation, pregnancy, ovulation, endometriosis
Who is at Risk?

- Increased risk based on: personal history, family cancer pedigree, known mutation carrier, prolonged use of infertility Rx

NOCEDP Clinical Experience

- Formal Genetic evaluation and Testing
- 3D US and Microvascular Index (MVI)
- Physical examination q 6m
- Health Services, QOL, Education
- Ovarian Pap Test- outpatient 0.9 mm miniscope
- Biomarkers unique to ovarian carcinogenesis, invasion, metastasis

Clinical Risk Assessment

- Nulliparity???? (92% parous > 1 child)
- Personal and Family History- critical
- Ashkenazi descent ? (why me?)
- 38% Jewish women with ovarian carcinoma- + BRCA 1 or 2
- 20% Jewish women with premenopausal Breast carcinoma- + BRCA 1 or 2
- All affected Jewish women should be offered genetic testing

What is Hereditary Cancer?

- 60% Sporadic
- 30% Familial
- 10% Hereditary

Hereditary Cancers

- BRCA2: 32%
- Other genes: 16%
- BRCA1: 52%

Who is at Risk for a Hereditary Ovarian Cancer Syndrome?

- Any person with a personal history or family history of:
  - Breast, Colon, or Uterine cancer diagnosis under the age of 50
  - Ovarian cancer diagnosed at any age
  - Male breast cancer at any age
  - Multiple primary cancers: (i.e. breast and ovarian cancer, bilateral breast cancer, ovarian and uterine cancer, colon and uterine cancer)
  - 2 or more family members with the same or related cancer diagnosis in the family
  - Ashkenazi Jewish ancestry (with breast or ovarian cancer)
  - Relatives of mutation carrier

A BRCA Mutation Increases Breast and Ovarian Cancer Risks

- Breast cancer by age 50: 2%
- Breast cancer by age 70: 7%
- Ovarian cancer by age 70: <2%
- General Population: Up to 44%
- BRCA Mutation: Up to 87%
A BRCA Mutation Increases Risk of Second Cancer

Risk of Cancer (%)

- BRCA Mutation
- General Population

- Ovarian Cancer
- Breast Cancer after 5 yrs
- Breast Cancer by age 70

0 10 20 30 40 50

Risks in Men With a BRCA Mutation

Risk of Cancer (%)

- General Population
- BRCA Mutation

- Breast Cancer
- Prostate Cancer

<1% 7% 7%
20%

Management/ Surveillance

<table>
<thead>
<tr>
<th>Breast Screening</th>
<th>General Population</th>
<th>High Risk</th>
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<tbody>
<tr>
<td>Self Breast Exam</td>
<td>Monthly beginning at age 18</td>
<td>Monthly beginning at age 18</td>
</tr>
<tr>
<td>Clinical Breast Exam</td>
<td>Every 1-3 yrs beginning at age 20 and yearly at age 40</td>
<td>2-4 x/y beginning at age 20</td>
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<tr>
<td>Mammogram</td>
<td>Yearly beginning at age 40</td>
<td>Yearly beginning at age 25-35</td>
</tr>
<tr>
<td>Breast U/S and/or MRI</td>
<td>PRN</td>
<td>Yearly (6 months after mammogram) beginning at age 25</td>
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Management/ Surveillance

<table>
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<th>Other Screening</th>
<th>General Population</th>
<th>High Risk</th>
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<tbody>
<tr>
<td><strong>Ovarian</strong></td>
<td>Not routinely recommended</td>
<td>Concurrent transvaginal ultrasound with color Doppler, pelvic exam, and CA-125 2x/yr</td>
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<tr>
<td><strong>Colon</strong></td>
<td>Colonoscopy every 5-10 years beginning at age 50</td>
<td>Colonoscopy every 3-5 yrs beginning at age 40</td>
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Management/ Surveillance

- **Surgical Options**
  - Prophylactic Bilateral Salpingo-Oophorectomy
    - Reduces the risk for Ovarian cancer by 96%
    - Reduces the risk for Breast Cancer by 50-68%
  - Prophylactic Mastectomy: Reduces the risk for breast cancer by 90%
- **Tamoxifen**
  - Affected: reduces contralateral Br ca by 75%
  - Unaffected: BRCA2: 62%; High Risk: 45%
- **Oral Contraceptives**: Reduces the risk for Ovarian Cancer by 60% (if used for >5yrs)

Other Hereditary Cancer Syndromes Associated with Breast or Ovarian Cancer

- **HNPCC**: Hereditary Non-Polyposis Colorectal Cancer:
  - Colon Cancer (75%); Endometrial (39%); Ovarian (5-10%); Others- GI area
- **Li-Fraumeni**:
  - Leukemia; breast; brain tumor; adrenocortical carcinoma; bone and soft tissue sarcoma; early onset adenocarcinomas or other childhood cancers.
- **Cowden**:
  - Breast, thyroid, endometrial
REVIEW of RED FLAGS FOR HBOC

- Early age onset breast cancer
- Bilateral breast cancer or both breast and ovarian cancer in same individual (regardless of age)
- Both breast and ovarian cancer occurring in one family regardless of age
- Member of BRCA mutation family
- Ashkenazi Jewish

Ovarian Cancer Syndromes

- Site-specific ovarian
- Breast-Ovarian
- Lynch type II - hereditary nonpolyposis colorectal cancer (HNPCC) – 9-12%
- Mutations of unknown significance
Risk Assessment

• Formal pedigree analysis and genetic testing and counseling by a team including board certified geneticists and gynecologic oncologists identified 981 women
• 919 BRCA1/2 +, 62 + pedigree assessment
• Prophylactic surgery consisted of a laparoscopic BSO, peritoneal washings, and comprehensive evaluation of pelvis and abdomen

Demographics

• 981 High Risk women
• 517 BRCA 1 + (55%)
• 402 BRCA 2+ (39%)
• 62 BRCA- (5%) yet pedigree c/w Inherited Cancer Syndrome
• Evaluation from 1990-present
• Average Clinical follow-up 5 years

BRCA 1

• 517 women
• 7 Gynecologic malignancies
  – PPC- 2- Stage IIC and IIIB
  – FT- 2- Stage IIB and IIIB
  – OVCa- 3- 1-Stage IA, 2-IIIA

  – No PPC in all women s/p BSO
BRCA 2

- 402 women
- 3 Gynecologic Malignancies
  - PPC- 1 Stage IIIB
  - FT- 1 Stage IIIA
  - OVCA- 1 Stage IB

- No PPC in women s/p BSO

Cancer Detection

- 971 Benign
- 3 Primary Peritoneal Cancer- all Stage III
- 3 Fallopian Tube Cancer- Stage II/III(2)
- 4 Ovarian Cancer- 2- Stage I, 2- Stage III
- 10 Cancers –
  - 2- Stage I
  - 1- Stage II
  - 7- Stage III

The Role of US

Best Visualization of the Fetus and Adnexa
Recent Advances In Ultrasound

• Power Doppler Energy- improved specificity as secondary test (83-92%)
• 3-Dimensional volume acquisition and power Doppler- identifies architectural and vascular changes in observed mass, increases specificity from 54% to 75% as a secondary test
• Microvascular Imaging (MVI)- capillaries visualized with nanoparticles

Complex adnexal mass with multiple septations with central flow suggestive of malignancy serous cystadenocarcinoma - confirmed by pathology

NOCEDP

- 47,356 gynecologic U/S on 13,646 asymptomatic high-risk women (normal exam and U/S)
- 297 aberrant masses identified
- 127 surgical interventions
- 113 benign tumors, 14 cancers

AJOG 2005
**NOCEDP**

- 14 asymptomatic gynecologic cancers detected (4 fallopian tube, 4 primary peritoneal, 2 epithelial ovarian carcinoma, 2 uterine, 2 metastatic Br)
- all Stage III/IV (A, B, and C) except uterine (both stage1A G1)
- all normal US and PE 12 and 6 months prior to abnormal scan
- FT/PPC - normal ovaries

**Conclusion**

- US was effective in detecting asymptomatic advanced stage adnexal disease
- US is ineffective as an independent modality in the detection of early stage EOC in the high-risk population

**The Future: Microvascular Imaging**

- Combination of high resolution ultrasound with vascular mapping and quantification of aberrant capillary influx from pre-existing host venules stimulated by tumor neovascularization
- IV contrast agents (micro- and nanoparticles) to illuminate the extravasation associated with the influx of new “leaky” vessels
Demonstration of normal ovarian vascularization (left) as compared to a morphologically “normal” ovary with Stage I EOC (papillary serous) (right). Note the significant increase in tumor vascularity as well as RBC extravasation throughout the parenchyma, both of which were perfused by contrast agents and therefore detected by contrast sonography. (H&E stain, x200 magnification)

Sonographic contrast agents can diffuse into the leaky capillary beds associated with tumor neovascularization which translates into enhanced sonographic visualization of early stage EOC.

In vivo model demonstrating sonographic detection of aberrant tumor vascularity. Time activity curve showing time to peak and full-width half maximum points used to quantify perfusion.
Mass Spectrometry as a Discovery Tool for Cancer Biomarkers

- Low resolution mass spectrometry profiles segregate cancer from non-cancer control
  - Sensitivity 100% and specificity 95%
- Low molecular weight archive
- Small proteins associate with high abundance carrier proteins
- Extraction and analysis of carrier-bound peptides a rich source of novel biomarkers

Validation and Implementation of SELDI-QqTOF for Diagnostic Proteomics

- Widely accessible
- Extensive m/z range (5-300,000)
- Low Resolution (~ 100-200)
- Low Mass Accuracy (~ 1000 ppm)

- Ciphergen SELDI-TOF MS
- EDRN 2002
- More specialized knowledge required...
- Limited m/z range? (5-12,000 – XL to 40,000)
- High resolution (~9000 at m/z 1500)
- High mass accuracy (~ 50 ppm - external cal)
- Able to conduct CID of peptides
Comparison of the mass spectra from control serum prepared on a WCX2 Protein Chip array and analyzed with a PBS-II TOF (panel A) or a Qq-TOF mass spectrometer (panel B).

prOTOF orthogonal high resolution MALDI TOF detector

- Mass accuracy:
  - 5-10 ppm external calibration
  - 2-5 ppm internal calibration
- Mass stability: 10 ppm over several hours using external calibration
- Resolution: 10,000-15,000
- Detection limits: sub femtomole
- Throughput: 15,000 samples/day
- Independence of operating parameters
- Disposable sample plates

The Rapid Evolution of MS Instrumentation

- Year 2002—Low Resolution SELDI-TOF
- Lancet 2002
- Year 2004—High Resolution SELDI-TOF
- ERC 2004
- Year 2005—Ultra High Resolution Orthogonal MALDI-TOF and FT-ICR
- JNCI 2005

The Rapid Evolution of MS Instrumentation
Rapid Identification of Biomarkers in Ovarian Cancer Serum Samples:

- Carrier protein-bound affinity enrichment
- High resolution MALDI OTOF MS
- Decision rule analysis and FTICR de novo sequencing

**Biomarker Amplification and Harvesting by Carrier Molecules**

**Albumin-Bound Peptide Purification and Biomarker Identification**

- Albumin trap and elution of bound proteins/peptides
- 1D protein gel separation and digestion
- In-gel tryptic digestion and excision gel lanes
- μLC MS/MS analysis → Data analysis and repetitive sequencing
De novo sequencing of putative marker peptides...

Accurate mass, isotopically-resolved, differentially-expressed BioXPRESSION processed peptides are selected for LC-MS/MS

Peptide Identification from Microcapillary Reverse-phase Tandem MS

- Data analysis through the Sequest Bioworks Browser
  - European Bioinformatics Institute non-redundant proteome set
    - Swiss-Prot
    - EMBL
    - Ensembl
- Mascot version 1.9.0 and NCBI BLASTCLUST

Protein Identification

- Over 800 distinct proteins identified in data set
- 618 previously unknown to exist in human sera
- Stage-specific proteins identified
  - 215 unique to Stage I
  - 127 unique to Stage III and IV
  - 30 found in both early and late stage
- 96 of novel proteins identified by ≥ 2 peptide matches
  - Indicates greater than 99% confidence of correct identification
Prominent SELDI-TOF ionic species (m/z 6631.7043) identified to correlate with the presence of ovarian cancer were amplified by albumin capture

- **Albumin Binding Fragments:** Ovarian Cancer
  - F-3-8 cyclo-PRO-glycophorin e
  - A Chain A, Crystal Structure of Human Apolipoprotein A
  - A Chain A, Transthyretin (Prealbumin)
  - A1AG Human Alpha 1 Acid Glycoprotein 1 Precursor (AGP 1)
  - A1AT Human Alpha-1-Antitrypsin  Precursor, Alpha-1-Protease Inhibitor, Alpha-1-Antiproteinase
  - A1BG Human Alpha 1B Glycoprotein
  - A2MG Human Alpha 2 Macroglobulin Precursor
  - ADP-Ribosylation Factor Binding Protein 3, golgi localized gamma ear containing, ARF binding protein 3, KIAA0154 gene product
  - AF070710 envelope glycoprotein (Human Immunodeficiency Virus Type 1)
  - AF077721 Pol Protein (Human Immunodeficiency Virus Type 1)
  - AF094250 envelope glycoprotein (Human Immunodeficiency Virus Type 1)
  - AF099171 protease (Human Immunodeficiency Virus Type 1)
  - AF190128 Rev (Human Immunodeficiency Virus Type 1)
  - AJ228172 gp120 (Human Immunodeficiency Virus Type 1)
  - ALC1 Human Ig Alpha-1 Chain C Region
  - Alpha 1 & 2 Hemoglobin, HBA Human Hemoglobin Alpha Chain
  - APA1 Human Apolipoprotein A-I Precursor, Apolipoprotein A-I
  - APA2 Human Apolipoprotein A-II Precursor, Apolipoprotein A-II
  - APC1 Human Apolipoprotein C-I Precursor, Apolipoprotein C-I
  - APC3 Human Apolipoprotein C-III Precursor, Apolipoprotein C-III
  - LPHUB apolipoprotein B-100 precursor – human
  - apolipoprotein D, apoD (human, plasma)
  - apolipoprotein E (homo sapien)
  - ASNS Human Asparagine Synthetase (glutamine hydrolyzing), TS11 Cell Cycle Control Protein
  - AT Human Alpha-1-Antitrypsin (Internal Fragment)
  - B Chain B, Crystal Structure of A Human Fcg
  - B Chain B, Crystal Structure of S-Nitroso-Nit
  - BBHU CFAB Human Complement factor B Precursor, C3/C5 Convertase, Properdin Factor B, Glycine Rich Beta Glycoprotein GBG
  - BRS3 Human Bombesin Receptor Subtype 3, Uterine Bombesin Receptor
  - C Chain C, Human Serum Transferrin, Recombinant
  - C1HUQB Complement Subcomponent C1q Chain B Precursor
  - C4HU complement precursor (validated)
  - Ceruloplasmin, Ferroxidase Human
  - CFA1 Human Complement Factor I Precursor, C3B
  - Clusterin, Complement Cytolysis Inhibitor (CLI), SP-40, Sulfated Glycoprotein 2, testosterone Repressed Prostate Message 2JNCI-2005, JBC-2005
  - Zinc finger Zinc finger homeobox Protein
  - Zinc finger homeobox Protein Rho GDP–dissociation inhibitor 1
  - CHORD containing protein 1
  - Vascular non-inflamator molecule 2 RB associated factor 600
  - cGMP gated cation channel UNCC transmembrane receptor 2
  - UNC5C UNC5C transmembrane receptor 2
  - Proto-oncogene TK
  - Proto-oncogene TK Centrosomal protein 2
  - Ubiquitin-activating enzyme E1
  - Ubiquitin-activating enzyme E1 Phosphodiesterase gamma
  - Caspase recruitment protein 12
  - Tyrosine protein kinase CSK
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Conclusions

- High resolution mass spectral analysis of albumin-enriched serum fraction effectively segregates while Microcapillary HPLC tandem MS identifies novel biomarker candidates
  - Normal from malignant, Early from late stage disease
  - Disease recurrence
  - Optimization of chemotherapeutics-chemosensitivity assays

Clinically Relevant Biological Markers for Early Detection

- Lysophospholipids (LPA) - LC/MS/MS
- Growth factors (p110, p60)
- Proteases (MMPs, Kallikreins)
- Proteomics - SELDI/ MALDI-TOF, ABI QqTOF, ESI-MS
- Many other proteins ... Prolactin, Leptin, Osteopontin, IGF-II...

Ovarian Pap Test

- Minimally invasive office laparoscopy - outpatient procedure
- Genomics and proteomics can detect precancer/cancer years before cytology - Prevention

Cont Ob/Gyn 2003, NEJM 2003
Summary of array CGH analyses of 54 serous ovarian cancers. Data are represented from 1pter (left) to 22qter and X (right). Vertical lines indicate chromosome boundaries. Red spots indicate regions that are homozygously deleted or highly amplified in some tumors. Regions most frequently increased in copy number are apparent on chromosomes 3q26 (EVI1) and 8q24 (MYC).

FISH assay: EVI1 and MYC for EOC detection

BRCA1+ Mutation with Normal Cytology

Normal histology

Prophylactic BSO

Abnormal Copy Number of EVI1 and MYC
New Paradigm for Cancer Detection

Asymptomatic Patient

↓

Serum/Plasma Protein and Lipid Analysis

↓

Diagnostic Imaging-US

MVI

↓

Ovarian Pap Test/ Biopsy- Cytopathology- Gene/Protein Profile

↓

Detection of Early Stage Disease

Collaborating Institutions


Australia, Israel, Hungary, Canada, Japan, Sweden, Finland, Germany, Holland