Rapid Assessment of Breast Cancer for Treatment Decisions

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ASCP Innovations in Pathology Service for LMIC
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Breast Cancer Care

- Prevention
- Early detection
- Diagnostics
- Therapeutics

Requirements For Rapid Breast Cancer Diagnosis

- Provide an accurate invasive carcinoma diagnosis
- Obtain prognostic factors that determine treatment

For LMIC and point-of-care testing:

- Unskilled technicians
- At reasonable cost
- In a timely manner

Goals:
1. To develop a diagnostic workflow that has utility in developing countries where need is greatest
2. To expedite and decrease cost of diagnosis in developed countries
Invasive Breast Carcinoma

Mammogram  Gross Pathology  Microscopic Pathology

Invasive Carcinoma Types

- Tubular
- Lobular
- Micropapillary
- BRCA1
- Metaplastic

- Unique patterns of spread and clinical outcomes
- ~20 different special types
- 75% are invasive ductal carcinoma of no special type

Traditional Prognostic Factors

1. Tumor special type
2. Estrogen & Progesterone receptor protein expression and HER2/neu gene amplification
3. Tumor grade (how rapidly it grows)
4. Tumor size
5. Spread to lymph nodes (metastases)

- Used in algorithms for determining probability of survival and benefit from chemotherapy and hormone therapy treatments

From Patient To Diagnosis

Obtaining the traditional prognostic factors

1. Take a core biopsy specimen of the tumor
2. Tissue processing to produce an H&E glass slide
3. Tissue testing for prognostic markers: ER, PR and HER2

Obtaining the tissue sample

Fine Needle Aspirate - Cytology

- Sub-optimal
  - High false negative rate (3-5%)
  - Benign findings in a palpable mass
  - High false positive rate (0.5-2%)
  - Benign reactive cells & atypical hyperplasia
  - In situ carcinoma
  - No tumor grading possible

- Cheap
  - Malignant diagnoses are better than no diagnosis for an ulcerating breast mass

http://www.thedoctors.com
Breast-Biopsy-and-Fine-Needle-Aspiration malpractice stats
Optimal sample type for an accurate cancer diagnosis
- Preserves tissue architecture
- Essential to distinguish non-invasive from invasive carcinoma

Relatively expensive procedure for LMIC
- ~$1500 for a reusable biopsy gun
- ~$50 per needle

Obtaining the tissue sample
Core Biopsy

Tissue Processing

Labor intensive, time consuming and very expensive
- Tissue preserved with formalin
- Dehydration and infiltration with paraffin wax to make a block
- Thin slices cut from paraffin block
  - Placed on a glass slide
  - Stained with Hematoxylin & Eosin
  - To visualize under a microscope

Tissue Processing

Cost and lack of skilled labor is a significant barrier preventing many hospitals having anatomic pathology services in LMIC

BWH, Partners In Health & Rwanda MOH collaboration
- ~$500,000 equipment cost setting up a tissue processing lab in Butaro Hospital, Rwanda
- ~$30,000 per year to run (consumables)
- 2 Rwandan technicians trained at BWH in Boston perform grossing, embedding, sectioning and staining of tissue

Slide Review Under A Light Microscope

Pathologist confirms tumor type and grade

Grade 1
- Well differentiated
- 97% survival at 15 yr
- Grade is how rapidly it grows (mitotic rate) and how similar it looks to normal breast glands
- Grade is prognostic for survival
- Poor tissue fixation and processing impairs tumor grading

Grade 3
- Poorly differentiated
- 70% survival at 15 yr
-...

Laser Scanning Confocal Microscopy

An alternative to tissue processing and glass slide light microscopy

- Optical sectioning with a laser through fresh tissue to produce digital images
  - 5 μm thick optical sections with 1 μm resolution will mimic traditional histology sections
  - Ideally need to image at a depth of 500 μm into the tissue

Imagine...

- an alternative to expensive and poor quality tissue processing
- not requiring skilled technicians and equipment to make paraffin blocks and H&E stained glass slides
Laser Scanning Confocal Microscopy

- Utility for routine clinical diagnosis is limited by penetrable depth and small size of scanned field at high resolution
- New microscope designs and post-imaging processing are making this technology feasible for diagnostic use

MIT Prototype Confocal Microscope

One example when cost is no barrier...

- Femtosecond pulsed laser (> $250,000)
- Bimodal imaging (fluorescent dye for nuclei and collagen reflectance)
- Optically section ~50 μm into the tissue, take large number of mosaic images and stitch together with post-imaging processing, then convert image to mimic H&E stained slide (purple nuclei and pink collagen)

Unimodal Confocal Imaging of Breast Cores

Vivascope 2500
- Point scanning beam, laser confocal microscope
- ~$80K to buy
- 2 mins to scan each image

Limitations:
- Unimodal imaging lacks cellular detail
- Need to mosaic images together
- Resolution not good enough for accurate diagnosis and grading
- Still too expensive

Tri-modal imaging is much better....

- Acridine orange (purple nuclei) and eosin (pink), and endogenous collagen reflectance (pink) improves image quality, better mimicking hematoxylin and eosin stained tissue

Skin, invasive carcinoma
Comparing deluxe with basic...

Confocal microscope  Multiphoton microscope  H&E

- Zeiss LSM510 (old confocal microscope design; $430K new, $70K second hand)
- Image quality when scanning breast tissue is comparable at both 10x and 20x

Limited depth, reasonable speed of scanning

- Depth: ideally 500μm, but only 25μm
- Imaging plus mosaicing speed for the confocal:
  - 2m/s/mm² at 20x
  - A core is ~200mm² = 20 mins per core

Technological Advances in Slide Microscopy

- Whole slide scanning
  - To make high resolution (400x) digital images in 2 minutes
  - Not efficient for high volume daily workload in US hospitals
  - Excellent model for use in Africa
- Telepathology
  - Pathologist remote from location of slide provides diagnosis based on digital images or whole slide images
- Computer-assisted Image analysis for diagnosis

Alternate microscope designs could cut costs and speed up scanning...

Line-Scanning, Stage Scanning, Confocal Microscope

Prototype Under Development
- Stationary laser beam with mobile stage reduces costs compared with scanning laser
- No mosaic knitting together of images required
- Simple, cheap, continuous wave diode laser used with tri-modal imaging
- Projected cost when commercialized: $10 000
- Will be very robust but likely limited by resolution - simple cases will be diagnostic

Computer Assisted Image Analysis

- Automated ER, PR, HER2 and Ki-67 reading
- Used world-wide
  - Publically available web-based app:
    - ImmunoRatio
      - For ER, PR and Ki-67
    - ImmunoMembrane
      - For HER2
- Making the cancer diagnosis
  - Finding diagnostically relevant areas on slide
  - Detecting presence of epithelial cells and nuclei
  - Identifying lymphocytes
  - Grading of breast carcinoma
    - Nuclear pleomorphism
    - Counting mitoses
    - Assessing tubule architecture
  - Benign versus malignant
    - UDH vs ADH and DCIS
  - Prognostic factors in stroma

### Tissue Testing for Prognostic Markers

**Estrogen and Progesterone Receptor**

**Background:**
- Estrogen Receptor positive disease (70%)
- Hormonal therapy with tamoxifen or aromatase inhibitor
- Decreases recurrence rate at 5 years by ~50%

**Tissue Testing for Prognostic Markers**

- **Immunochemistry on glass slide sections of tumor tissue detects protein**
- **Reporting:**
  - Read by a pathologist
  - Positive threshold ≥ 1% tumor cells

**Response to hormonal therapy:**
- 75% ER + PR +
- 30% ER + PR -

### HER2/neu 17q12 Gene Amplification

**HER2 Background:**
- Membrane-bound growth factor receptor
- 15% breast tumors amplified
- 50% respond to HER2 targeted therapy
  - Antibody therapy or tyrosine kinase inhibitor

**Tissue Testing for HER2/neu 17q12 Gene Amplification**

- **Performed in a reference lab and read by a pathologist/skilled technician**
- **Immunohistochemistry (protein)**
- **In Situ Hybridization (DNA)**
  - Fluorescent or brightfield ISH
  - Amplified FISH ≥ 6.0 HER2 signals/cell
  - Or HER2/CEP17 ≥ 2.0

### Alternative POCT Assays for ER, PR and HER2

**Many years have been spent optimizing current protein and ISH assays for accuracy and quality**

- These tests require skilled personnel to perform and read them, expensive lab equipment, and take around 6hr to obtain a result
- An alternative is measuring mRNA levels in the tumor
  - Intermediate step between DNA and protein expression

### Gene Expression Profiling

**measuring many different mRNA levels in tumors at once**

- Correlate with protein levels for ER, PR and HER2
- Identify many molecular subtypes of breast tumor
  - ER/PR +ve, HER2 +ve and Triple Negative
- Provide tumor grade
  - Single or multiple genes
- Predict response to chemotherapy and prognosis
  - OncotypeDX, Mammaprint, Breast Cancer Index
Why don’t we routinely use mRNA levels of prognostic markers?

- **Cost:**
  - Immunohistochemistry (IHC) used to be cheaper than measuring mRNA levels

- **Accuracy:**
  - Reading a protein immunostain involves looking at tissue architecture and prevents errors
  - Easily distinguish in-situ carcinoma, invasive carcinoma and normal breast epithelium

A Successful Breast Cancer mRNA Assay...

- Only one mRNA assay, OncotypeDx, has gained any traction in routine breast cancer care
  - The OncotypeDx 21 gene assay predicts distant recurrence risk and survival benefit from chemotherapy
  - Reports single gene mRNA ER, PR and HER2 levels

mRNA Doesn’t Always Correlate with Protein

OncotypeDX mRNA data for ER and PR

- ER is 98% concordant, PR is 91% concordant with protein immunohistochemistry results
- In 70% of discordant cases, the protein immunohistochemistry score is correct result


mRNA Doesn’t Always Correlate with Protein

OncotypeDX mRNA data for HER2

- 36 HER2 positive cases by both IHC and FISH
- 39% (14/36) reported as negative
- High false negative rate has been a concern for mRNA profiling of HER2


Prognostic Marker mRNA Profiling

- No mRNA ER, PR, HER2 assay currently commercially available in the US

- Assay under development from Cepheid uses the GeneXpert
  - RT-Q-PCR machine
  - Already widely utilized in developing countries
  - >15,000 DX in 182 countries
  - STRAT 4 mRNA-pairing of ESR1, PGR, ERBB2, MKI67

Cepheid GeneXpert RT-Q-PCR Machine

- Dedicated cartridges test for infectious diseases (especially TB) and cancer diagnostics
  - 23 CE-marked tests & 17 FDA approved tests
- Relatively affordable
  - ~$17,000 per GeneXpert IV instrument in LMIC
  - < $3000 for GeneXpert OMNI one cartridge instrument
- Simple to use
  - combines sample preparation with amplification and detection in one with results in ~2hr
Cepheid Breast Cancer mRNA assay for ER, PR, HER2 and Ki-67

- Concordance with immunohistochemistry ER, PR and HER2 results is good in several datasets (unpublished)
- Recently tested scope of use
  - Limitations of the test
  - Minimal invasive tumor size and cellularity
  - Predictable false-positive and false negative scenarios
  - Samples fixed and processed in Rwanda

Study intended to challenge the assay limits

Concordance between protein IHC and mRNA assay:
In tissue processed at BWH (n = 83)

- ER: 95%
  - 80% accurate ER-ve tumors with background ER+ve normal epithelium
  - 91% accurate in small area tumor samples spanning ≤5x5mm (≤25mm²)
- HER2: 94%
  - 99% if intentionally difficult cases removed
  - HER2+ve DCIS adjacent to HER2-ve invasive
  - HER2 2+ IHC with low level amplification: HER2 copy numbers of 6.0-7.9
- Ki-67 (surrogate grade marker) with mitotic score: 92%
  - lo/hi cut point 8.2 mitoses/mm²

Data presented in poster SABCs 2016

Study using Rwandan processed samples

Concordance between protein IHC and mRNA assay:
In tissue processed in Rwanda (n=141)

- ER: 93%
  - lower than in previous data-sets, possibly due to tissue cold ischemic time impairing mRNA stability ("false-negative" mRNA results)
  - 5/6 ER-IHC discrepant results were confirmed concordant with repeat IHC using alternative antibody (6F11 vs SP1)
- HER2: 98%
  - Excellent data:
    - 2/3 HER2 discordant cases were confirmed concordant with mRNA results by FISH
    - 5/10 equivocal IHC calls had mRNA results concordant with FISH

Data presented USCAP 2017 Poster VI:454

Use of the Xpert Breast Cancer STRAT4 mRNA assay

Lessons learned:
- No need to macrodissect tumor routinely
  - Normal breast epithelium doesn’t give a false positive
- Predictable ER discrepancies:
  - Mostly in small volume tumors ≤50mm²
  - Low level ER expressing tumors (either weak or low %)
- Predictable HER2 discrepancies
  - HER2+ve DCIS admixed with HER2-ve invasive
  - Low level amplified tumors with average HER2 copy number 6-7

Next Step – Clinical trial in Butaro Hospital, Rwanda

Advantages of using GEP for prognostic information

Currently possible:
- ER, PR, HER2
- Genomic grade
- Many molecular subtypes with prognostic significance
- Chemotherapy response prediction

In the future:
- All histologic special subtypes
- Predictive signatures for hormonal therapy and specific chemotherapy sensitivity and resistance

Challenges of using GEP for prognostic information

- Differentiating in situ from invasive disease
  - High grade in situ and invasive molecular signatures are indistinguishable
  - Still requires visualization of tissue architecture under a microscope
  - Could be computer assisted diagnosis in unskilled hands
- Tumor size
  - Cannot be predicted by mRNA expression profiling
  - Imaging is mostly accurate
  - Important for surgery decision making
  - Less important for deciding treatment beyond surgery
Challenges of using GEP for prognostic information

- Lymph node status
  - Cannot be predicted by mRNA expression profiling
  - Extent of axilla node involvement dictates axillary treatment options

- Sentinel lymph node sampling
  - GEP can identify tumor cells present in node tissue
  - Quantification required to correlate with outcome and maintain prognostic value of sentinel node sampling
  - GEP not recommended in US

Diagnosis of breast cancer is not a rapid diagnosis at point-of-care practice today

It is possible to transform breast cancer diagnosis

The Potential Pathway For Rapid Breast Cancer Diagnosis
- in LMIC

- Affordable confocal microscope for optical imaging of core biopsies in hospitals lacking anatomic pathology dedicated resources
  - Thousands of hospitals worldwide

- Image analysis software to assist in easy diagnoses
  - Guide tissue allocation by an unskilled technician

- Fully automated, high quality mRNA prognostic marker and tumor grade testing

- Telepathology for difficult diagnoses of digital images by a pathologist remote from the testing site

The Potential Pathway For Rapid Breast Cancer Diagnosis
- in our well-funded hospitals

Current Practice
- 24-72hr for cancer diagnosis, tumor grade and prognostic markers, ER, PR, HER2

Rapid Testing Potential
- 30 min – with confocal microscopy of fresh core biopsy to confirm invasive carcinoma present, +/- grade of tumor
- 2hr - fresh (or fixed) tissue mRNA assay to identify prognostic factors (ER, PR, HER2 and tumor grade)

Why bother?
- Relieve patient stress and expedite treatment decision making
- Research benefits: core biopsies often taken prior to starting on a clinical trial and precious tissue not wasted by optical imaging

Thank you! Please contact me with questions or to collaborate jobrock@partners.org

Butaro Hospital, Rwanda