What is the Current Role of Biomarkers and Risk Stratification of Barrett’s Esophagus?

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Disclosures

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Consultant: Phathom Pharmaceuticals, Cernostics Inc, Interpace Diagnostics, CDx, and Isothive
Endoscopic Surveillance Strategies Are Ineffective

These data highlight the importance and need of developing biomarkers to detect neoplastic progression in Barrett’s esophagus.

Biomarker-Guided Surveillance Strategy: 50 Year-Old White Men with GERD Symptoms Followed 30 Years

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Cancers</th>
<th>Cancer Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>No surveillance</td>
<td>366</td>
<td>356</td>
</tr>
<tr>
<td>Dysplasia-guided surveillance</td>
<td>315</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>(↓14%)</td>
<td>(↓56%)</td>
</tr>
<tr>
<td>“Perfect” biomarker-guided</td>
<td>172</td>
<td>46</td>
</tr>
<tr>
<td>surveillance</td>
<td>(↓53%)</td>
<td>(↓87%)</td>
</tr>
</tbody>
</table>

Rubenstein et al. AP&T, 22; 135-146, 2005
Mutations that inactivate but stabilize p53 show overexpression.

Mutations that prevent translation show loss of expression.

Largest body of evidence as a biomarker
Easy and commonly used by pathologists

- p53 overexpression
- Aberrant p53 Expression
- Loss of p53 expression
Meta-Analyses of Risk for Neoplastic Progression in BE Based on p53 Immunostaining

- Studies that used p53 IHC in baseline Barrett’s biopsies, with follow-up evaluating neoplastic progression

Case-control and cohort studies show consistent, strong, and significant associations between aberrant p53 IHC and progression to high grade dysplasia or cancer.
Meta-Analyses of Risk for Neoplastic Progression in BE with LGD Based on p53 Immunostaining

- 2 studies exclusively enrolled LGD patients
  - Odds ratio was 21 in the case-control study
  - Relative risk was 5.7 in the cohort study

Scoring of p53 is subject to interpretation by the pathologist and there are no widely accepted standard criteria for “abnormal p53 staining”.

Specificity for p53 Immunostaining to Predict Risk

- p53 overexpression: Sensitivity 62%; Specificity 80%
- Loss of p53 expression: Sensitivity 31%; Specificity 98%

Abnormal TP53 Predicts Risk of Progression in Patients with Barrett’s Esophagus Regardless of a Diagnosis of Dysplasia

• Standardized criteria for abnormal p53 IHC were developed and validated in 233 Barrett’s patients (183 NDBE, 50 HGD)

• Cases: 179 patients developed HGD/EAC on follow-up ≥1 year later

• Controls: 179 patients did not develop HGD/EAC on follow-up ≥1 year later matched for age and sex

• Abnormal p53 IHC: sensitivity 49.7%%, specificity 93.8%, OR 58, and HR 5 for progression

• Prospective validation of these criteria for abnormal p53 IHC by a large, community-based pathology practice confirmed their ability to predict neoplastic progression in NDBE.

follow-up ≥1 year later matched for age and sex

• Abnormal p53 IHC staining was found > 5 years before the detection of dysplasia

Systems Biology

- Views tissue as a system with multiple compartments to be analyzed for genetic, immunologic, vascular, and morphologic features of neoplastic progression

- **TissueCypher™ (Cernostics)**
  - Uses formalin-fixed, paraffin-embedded Barrett’s biopsies
  - Analyses for a panel of biomarkers associated with Barrett’s neoplasia in epithelial, stromal, and immune cells

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Process Involved</th>
<th>Abnormality Indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>Tumor suppressor, apoptosis</td>
<td>Nuclear overexpression or loss in epithelial cells</td>
</tr>
<tr>
<td>p16</td>
<td>Cell cycle control</td>
<td>Cellular loss in epithelial cells</td>
</tr>
<tr>
<td>HER2/neu</td>
<td>Cell growth, proliferation</td>
<td>Plasma membrane overexpression in epithelial cells</td>
</tr>
<tr>
<td>AMACR</td>
<td>Lipid metabolism</td>
<td>Overexpression in peroxisomes and mitochondria of epithelial cells</td>
</tr>
<tr>
<td>COX-2</td>
<td>Inflammation</td>
<td>Overexpression in epithelial and stromal cells</td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophages</td>
<td>Stromal density and phenotype of macrophages</td>
</tr>
<tr>
<td>CD45RO</td>
<td>Memory lymphocytes</td>
<td>Stromal density</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Angiogenesis</td>
<td>Expression and subcellular localization in stromal cells</td>
</tr>
<tr>
<td>Cytokeratin-20</td>
<td>Metaplasia</td>
<td>Plasma membrane expression in epithelial cells</td>
</tr>
</tbody>
</table>
TissueCypher® For Predicting Risk of Neoplasia

Multiplexed Immunofluorescence Slide Labeling
9 protein-based biomarkers plus Hoechst labeling of nuclei

Whole Slide Fluorescence Scanning
4 registered channels of image data for each slide

CD45RO
Studies on TissueCypher® for Predicting Neoplastic Progression of Barrett’s Esophagus

5 independent studies of 239 progressors and 656 non-progressors at medical centers in US and Europe

Case-control studies show consistent, strong, and significant associations between a TissueCypher high-risk score and progression to high grade dysplasia or cancer and between a TissueCypher low-risk score and lack of progression to high grade dysplasia or cancer.

- NIH funded study (Cleveland Clinic & UPMC) that independently validated ability of TissueCypher to predict incident progression from ND, IND, and LGD to HGD/EAC (n=366)

- Demonstrated that TissueCypher also detects presence of prevalent HGD/EAC (n=30)

- NIH funded study (Cleveland Clinic & UPMC) that independently validated ability of TissueCypher to predict incident progression from ND/IND/LGD to HGD/EAC (n=268)

- Further validation of TissueCypher’s ability to predict incident progression in patients with non-dysplastic BE (n=76), and demonstrated that assessment of additional spatial and temporal biopsies increases TissueCypher’s sensitivity.

- Further independent validation of TissueCypher’s ability to predict progression patients with LGD (n=155)

Non-dysplastic Barrett’s patients who scored high-risk progress to high grade dysplasia or cancer at a rate higher than that with an expert pathologist-confirmed diagnosis of low grade dysplasia.

- Case-control studies show consistent, strong, and significant associations between a TissueCypher high-risk score and progression to high grade dysplasia or cancer and between a TissueCypher low-risk score and lack of progression to high grade dysplasia or cancer.

- Further independent validation of TissueCypher’s ability to predict progression patients with LGD (n=155)
Prediction of Progression in Barrett’s Esophagus Using a Tissue Systems Pathology Test: A Pooled Analysis of International Multicenter Studies

- 475 Barrett’s patients (403 NDBE, 43 LGD, 29 IND) from 4 independent case-control studies in the US and Europe
- **Cases:** 152 patients developed HGD/EAC on follow-up ≥1 year later
- **Controls:** 323 patients did not develop HGD/EAC on follow-up ≥1 year later matched for age and sex
- Age, sex, histologic diagnosis, BE segment length, and hiatal hernia used in a clinical prediction model
- High risk score: sensitivity 38.6%, specificity 93.8% and independently predicted a ~8-fold ↑ in progression
- The TissueCypher risk score significantly improved the predictive value of the clinical model
Imaging Biomarker: Wide-Area Transepithelial Sampling (WATS) with Computer-Assisted 3D Analysis

- Abrasive brush scrapes an extensive area of the Barrett’s segment
- Provides a thick tissue specimen
- Combination of AI neural network and 3D computer imaging of the tissue to identify abnormal cells for review by a pathologist.
- Obtain a cytology specimen for morphological analysis as well as for immunostaining for proteins such as p53

Courtesy of Dr. Rob Odze and CDx
The Addition of WATS to Forceps Biopsies Detects More Indefinite and LGD

- Community endoscopists at 21 sites enrolled 12,899 patients having screening or surveillance for Barrett’s esophagus
  - FB found dysplasia in 88 pts (0.68%), WATS added 213 pts (1.65%)
    - WATS increased dysplasia detection rate from 0.68% to 2.33%
    - 213 additional patients: 128 indefinite, 75 LGD, 10 HGD

ASGE Conditional Recommendation: In patients with known or suspected Barrett’s esophagus, we suggest using WATS-3D in addition to Seattle protocol biopsy sampling.

- WATS increased dysplasia detection rate from 2% to 5%
- 74 additional patients: 70 indefinite/LGD, 4 HGD

Smith MS et al. Dis Esoph 2019;32(3).
Tests of Genomic Instability For Predicting Neoplastic Progression

- **Genomic Instability**
  - ↑ genome alterations during cell division
    - Small structural alterations (base pair mutation, MSI)
    - Large structural alterations [chromosome number (aneuploidy), structure-LOH]
• **Mutational Load** (Index of genomic instability)
  – BarreGEN®
    • Pathologist microdissects targets on H&E slides
    • LOH mutations and MSI at 10 loci for tumor suppressor genes assessed using PCR and quantitative capillary electrophoresis of DNA
    • Mutational load (ML) quantified on a scale of 0-10
Mutational Load Predicts Neoplastic Progression

• Case-Control Study
  – 23 Cases: Patients with NDBE or LGD at baseline who developed HGD/EAC on follow-up ≥1 year later
  – 46 Controls: Patients with NDBE or LGD at baseline who did not develop HGD/EAC on follow-up ≥1 year later

Contradictory study results limit the utility of mutational load for prediction of neoplastic progression in Barrett’s esophagus.

- Subsequent Study – ROC curves showed poor discrimination of ML in predicting progression (AUC 0.50 at ML≥1)
  – Used crude lysates rather than purified DNA


Barrett’s Aneuploidy Decision: BAD

• Novel PCR-based method (RealSeq) to amplify genome-wide loci for sequencing allowing for the identification of single chromosomal arm gains or losses

• Performed on esophageal brushings

• Chromosomal arm level scores are integrated into a Global Aneuploidy Score (GAS)

• 6 specific chromosomal alterations - gain 1q, 12p, 20q and 8q24; loss 9p and 17p

• BAD Score: Global Aneuploidy Score + 6 chromosomal alterations
BAD Scores May Predict Neoplastic Progression

- Medical records were reviewed in a blinded fashion for 60 NDBE cases over a 36 month follow up period
  - 40 cases were classified as Not-BAD and none of these cases progressed to HGD/EAC
  - 16 cases were classified as Maybe-BAD and none of these cases progressed to HGD/EAC
  - 4 cases were Very-BAD and 2 of the 4 (50%) progressed

New, promising biomarker test that is easy to use (PCR-based) and can be performed on endoscopic brushings…

And the name is not BAD either!

Douville...Chak Gastro