Biomarker Based Non-Endoscopic Detection Of Barrett’s Esophagus (BE) And Esophageal Adenocarcinoma (EAC)

Sanford Markowitz
Helen Moinova
Joseph E. Willis
Amitabh Chak
• Sanford Markowitz receives royalties on methylated DNA technology licensed to Exact Sciences and is a founder, board member, consultant, and inventor on patents licensed to Lucid Diagnostics.
The Case Esophagus Team

Helen Moinova  Amitabh Chak  Joe Willis

Tom LaFramboise  Kishore Guda
Esophageal Cancer (EAC):

- Fastest growing cancer in U.S.
- 5-year survival < 20%
- Death toll now exceeds ovarian cancer
- Seldom detected early
Barrett’s esophagus (BE)

- Only known precursor to EAC
- Occurs in distal esophagus in patients with GERD
Barrett’s esophagus (BE)

- Only known precursor to EAC
- Occurs in distal esophagus in patients with GERD
- Detection of BE and ablation of dysplastic BE can prevent cancer
Challenges to BE Screening by EGD
Challenges to BE Screening by EGD

- EGD is costly, requires sedation, day off work.
- EGD Screening only recommended by AGA, and only in those with chronic GERD + additional risk factors.
- EGD screening, even in GERD, not generally accepted by primary care physicians or patients.
- Challenge: Antecedent BE recognized beforehand in <10% of EAC cases.
The Need

• Needed: Non-EGD Biomarker Driven approaches that can detect BE more easily and at less expense than EGD.
Biomarker Approach

Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett’s esophagus

Helen R. Moinova, Thomas LaFramboise, James D. Lutterbaugh, Apoorva Krishna Chandar, John Dumot, Ashley Faulx, Wendy Brock, Omar De la Cruz Cabrera, Kishore Guda, Jill S. Barnholtz-Sloan, Prasad G. Iyer, Marcia I. Canto, Jean S. Wang, Nicholas J. Shaheen, Prashanti N. Thota, Joseph E. Willis, Amitabh Chak, Sanford D. Markowitz


Non-Dysplastic Barrett’s Detection

Specificity: 92%
Sensitivity: 90%
Biomarker Approach

i) Methylated DNA Biomarker Panel

ii) Swallowable Esophageal Sampling Device
Vimentin Gene Methylation (mVim) is a Colon Cancer Biomarker

Methylated in 80% of Colon Cancer Cases
Vimentin Gene Methylation (mVim) is a Colon Cancer Biomarker

Methylated Vimentin DNA: Commercialized by Exact Sciences For Stool DNA Detection of Colon Cancer (ColoSure™, LabCorp)
Vimentin Methylation as a Gastrointestinal Cancer Biomarker Moinova et al.

Vimentin Methylation in Barrett’s/EAC (91% Sensitive)
Pilot Study – Molecular Cytology: 90% of BE Detected by mVIM DNA on Esophageal Brushings
Molecular Cytology: mVim in Esophagus Cytology Brushings (N=310)

Training set

AUC: 0.948
If >1.05%:
Sensitivity: 90.7%
Specificity: 93.2%
Cases: 107; Controls 59

Validation set

AUC: 0.956
If >1.05%:
Sensitivity: 91.5%
Specificity: 92.6%
Cases: 117; Controls 27
New Complementary Methylation Marker

- If One Marker is Good, Would Two Markers Be Better?
New Complementary Methylation Marker

• If One Marker is Good, Would Two Markers Be Better?

• Genome Wide RRBS in 72 Biopsies: 26 EAC/Sq pairs; 15 BE; 5 EAC Cell Lines
New Complementary Methylation Marker (mCCNA1)

**CCNA1 locus**

- NGS assay 176 bp, 21 CpGs
- RRBS patch, 90bp, 7 CpGs
- CpG island 872 bp, 103 CpGs

**mCCNA1**

Fraction of methylated reads

<table>
<thead>
<tr>
<th></th>
<th>Sq</th>
<th>NDBE</th>
<th>HGD</th>
<th>EAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Molecular Cytology: mCCNA1 in Esophagus Cytology Brushings (N=313)

Training set

mCCNA1

AUC: 0.954
If >3.12%:
Sensitivity: 90.7%
Specificity: 98.4%
Cases: 108; Controls 61

Validation set

mCCNA1

AUC: 0.952
If >3.12%:
Sensitivity: 89.6%
Specificity: 92.9%
Cases: 115 Controls 28
### Molecular Cytology: mVIM + mCCNA1 in 313 brushings

<table>
<thead>
<tr>
<th></th>
<th>mVIM (Positive if &gt; 1.05%)</th>
<th>mCCNA1 (Positive if &gt; 3.12%)</th>
<th>Either mVIM or mCCNA1 Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control GEJ</td>
<td>93%</td>
<td>97%</td>
<td>91%</td>
</tr>
<tr>
<td>(GERD, EE,</td>
<td>86</td>
<td>89</td>
<td>84</td>
</tr>
<tr>
<td>other)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDBE</td>
<td>92%</td>
<td>80%</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>69</td>
<td>72</td>
</tr>
<tr>
<td>LGD</td>
<td>94%</td>
<td>91%</td>
<td>94%</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>HGD</td>
<td>87%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Cancer</td>
<td>91%</td>
<td>95%</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>99</td>
<td>100</td>
</tr>
</tbody>
</table>
Additional Specificity Studies

Smoking associated DNA methylation in proximal esophagus brushings

mCCNA1

$p=0.0094$

mVIM

$p=0.0155$
The Challenge

• Comfortable Non-Endoscopic Sampling of the Distal Esophagus / GE Junction

• Without Sampling the Proximal Esophagus and Throat
JASSS Balloon

Joe/Amitabh/Sandy’s Swallowable Sampling Balloon

Capsule sized balloon

Tethered balloon is swallowed

Balloon with structured surface:
   i) inflated in the stomach
   ii) pulled back to sample distal esophagus

Balloon deflated for retrieval
JASSS Balloon – Commercialized as EsoCheck

YouTube Video

32 seconds
Study Design

• Patients with BE, EAC, or GERD controls were recruited for EsoCheck prior to EGD

• EsoCheck sampling was performed

• Balloons were placed in vials and frozen

• DNA was extracted from balloons, bisulfite converted, and sequenced with NGS

• A software pipeline calculated % DNA reads arising from bisulfite converted methylated versus unmethylated Vim and CCNA1 templates
Results - Tolerance

• Procedure took mean 3.1 minutes (range, 1 – 14 min).

• 72% of the subjects rated overall tolerance as excellent

• 93% preferred EsoCheck to EGD and were willing to repeat it again.

• Scores of 1 or 2 on a 10-point Likert scale, denoting little to no anxiety, pain, or choking, were reported by 75%, 95%, and 82% of subjects, respectively.
Performance Equals Endoscopic Brushings

- **mVIM**
  - AUC: 0.91
  - N=36 controls, 50 cases

- **mCCNA1**
  - AUC: 0.92
  - N=36 controls, 50 cases
## Performance in 86 Subjects

<table>
<thead>
<tr>
<th>Sample #</th>
<th>mVIM (Positive if &gt;1.0%)</th>
<th>mCCNA1 (Positive if &gt;1.0%)</th>
<th>Either mVIM Or mCCNA1 Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity on Controls</td>
<td>36</td>
<td>92%</td>
<td>100%</td>
</tr>
<tr>
<td>Sensitivity: Non-Dysplastic BE (&gt;1cm)</td>
<td>31</td>
<td>81%</td>
<td>71%</td>
</tr>
<tr>
<td>Sensitivity: All Dysplastic BE</td>
<td>11</td>
<td>73%</td>
<td>73%</td>
</tr>
<tr>
<td>Sensitivity: All Cases</td>
<td>50</td>
<td>80%</td>
<td>72%</td>
</tr>
</tbody>
</table>
CONCLUSIONS

• EsoCheck encapsulated balloon can sample the distal esophagus with excellent tolerability and acceptability.

• Methylated Vimentin and CCNA1 can be successfully assayed on EsoCheck samples by NGS bisulfite sequencing.

• BE and EAC detected with high sensitivity and specificity, demonstrating the feasibility of non-endoscopic unsedated BE screening.
**Eosophageal Carcinoma**

Esophageal Carcinoma caused an estimated **15,690 deaths** in the United States in 2017, and has a five-year survival rate of less than 20%

Barrett’s Esophagus is the precursor for Esophageal Carcinoma, but routine clinical visits do not check patients for this lesion

Esophageal Carcinoma is associated with a poor prognosis because the disease is typically diagnosed at late stages

Detecting Barrett’s Esophagus currently requires endoscopic evaluation, but a new DNA biomarker screening method and balloon-based sampling device could allow for quick and non-invasive diagnosis

---

**Endoscope diagnosis**

- Time-consuming  
- Patients must be sedated  
- Based on tissue characteristics  
- Expensive

**DNA Biomarker**

- Samples collected in less than five minutes  
- Patients fully conscious  
- DNA-sequence based  
- Inexpensive
How the Swallowable Balloon Device Helps Detect Barrett Esophagus

1. Swallow

Instead of undergoing standard endoscopy, patients can swallow a pill-sized capsule attached to a thin silicone catheter. The capsule passes through the esophagus and stops near the stomach.

2. Inflate & Collect

Once the capsule nears the stomach, a balloon with a textured surface is inflated and maneuvered to swab the lower esophagus, where Barrett esophagus (BE) typically begins. A sample of the cells lining the lower esophagus is collected.

3. Retrieve

The balloon is deflated through the catheter and inverted back into the capsule, thus protecting the sample from dilution or contamination. After retrieving the capsule through the mouth, DNA is extracted from the balloon's surface for a DNA methylation test.

Device capsule and catheter in comparison to a vitamin pill and a dime.

The small dimensions of the balloon device allow clinicians to retrieve samples quickly and easily without sedation during an outpatient exam.

Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett's esophagus


Non-Dysplastic Barrett's Detection

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>92%</td>
<td>90%</td>
</tr>
</tbody>
</table>
Introducing

EsoCheck

a five minute office based procedure to sample cells from the esophagus

LEARN MORE

ADVANCING POSSIBLE

Lucid Diagnostics is developing a revolutionary non-invasive, office based diagnostic test for biomarkers to esophageal cancer.
FDA 510K Approval for EsoCheck Balloon Device

Introducing

EsoCheck

a five minute office based procedure to sample cells from the esophagus
Introducing

EsoCheck

a five minute office based procedure to sample cells from the esophagus
Biomarker Assay (EsoGuard) Awarded FDA Breakthrough Designation

Introducing

EsoCheck

a five minute office based procedure to sample cells from the esophagus

LEARN MORE

ADVANCING POSSIBLE

Lucid Diagnostics is developing a revolutionary non-invasive, office based diagnostic test for biomarkers to esophageal cancer.
PLA Code for EsoGuard LDT Awarded

Introducing

EsoCheck
a five minute office based procedure to sample cells from the esophagus

LEARN MORE

ADVANCING POSSIBLE

Lucid Diagnostics is developing a revolutionary non-invasive, office based diagnostic test for biomarkers to esophageal cancer.
Current Status

• 2\textsuperscript{nd} Generation EsoCheck Balloon with Improved Swallowability and Increased DNA Collection

• Case Control Study to define optimal performance of New Balloon with Marker Panel

• Multi-center BE detection study in at risk screening population.

• Biomarker Discovery to Distinguish BE versus Dysplasias and Cancers to Enable Non-Endoscopic BE Surveillance
Thank You EDRN!

Introducing

**EsoCheck**

a five minute office based procedure to sample cells from the esophagus

LEARN MORE

ADVANCING POSSIBLE

Lucid Diagnostics is developing a revolutionary non-invasive, office based diagnostic test for biomarkers to esophageal cancer.
Molecular Cytology: mVIM + mCCNA1 in 313 brushings

mVIM and mCCNA1 in combined esophageal brushings from 229 cases of BE/EAC and 84 controls

- **mVIM**
  - AUC: 0.956
  - Cases: 230; Controls 86

- **mCCNA1**
  - AUC: 0.954
  - Cases: 229; Controls 89