Evaluation of POU4F3 methylation as a new biomarker of cervical precancer and cancer

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I am the Clinical Director of Neumann Diagnostics Ltd., Cellcall Ltd. and the Head of SYNLAB Genoid Molecular Diagnostic Laboratory at Synlab Hungary Ldt.

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Marta Benczik MD
Limitations of Cervical Cytology

Successful screening programs
- Organized cytology based screening programs
- Reduced cervical cancer incidence in industrialized countries

Limitations of cervical cytology
- Inter-observer variability, inadequate slides
- Modest sensitivity and low NPV, cost burdens
- Equivocal result, unnecessary treatment
Why to test HPV status in the first line?

‘of current cervical screening technologies, molecular HPV testing offers

by far best negative predictive value

for detection of high grade CIN’  
(Dillner et al, 2008)
Molecular Based HPV Detection

<table>
<thead>
<tr>
<th>Comparing to cytology</th>
<th>Questions of HPV testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>• High sensitivity</td>
<td>• Low specificity</td>
</tr>
<tr>
<td>• More reproducible</td>
<td>• Benign and self-limiting HPV infections</td>
</tr>
<tr>
<td>objective evaluation</td>
<td>• Sufficient risk stratification is needed</td>
</tr>
<tr>
<td>• QA/QC, automated, high-throughput</td>
<td>• HPV16/18 genotyping or reflex cytology</td>
</tr>
<tr>
<td>• High NPV, prolonged</td>
<td></td>
</tr>
<tr>
<td>screening intervals</td>
<td></td>
</tr>
</tbody>
</table>
The most recent guidelines recommend primary high risk HPV test, with genotyping and cytology triage (Huh et al 2015), or cytology triage alone (Karsa et al 2015).
**CONFIDENCE™ Marker**

Significantly higher sensitivity with comparable specificity

<table>
<thead>
<tr>
<th></th>
<th>Relative SENSITIVITY</th>
<th>Relative SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CIN2+</strong></td>
<td>1.67</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>95% CI: 1.23-2.24</td>
<td>95% CI: 0.7-1.22</td>
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<tr>
<td><strong>CIN3+</strong></td>
<td>1.74</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>95% CI: 1.25-2.33</td>
<td>95% CI: 0.66-1.21</td>
</tr>
</tbody>
</table>

n=1,287 CONFIDENCE™ HPV positive women aged 25-65

TRACE Study (total n=6,761), verification bias adjusted data
Performance of a new HPV and biomarker assay in the management of hrHPV positive women: Subanalysis of the ongoing multicenter TRACE clinical trial (n > 6,000) to evaluate POU4F3 methylation as a potential biomarker of cervical precancer and cancer

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The ongoing Triage and Risk Assessment of Cervical Precancer by Epigenetic Biomarker (TRACE) prospective, multicenter study aimed to provide a clinical evaluation of the CONFIDENCE™ assay, which comprises a human papillomavirus (HPV) DNA and a human epigenetic biomarker test. Between 2013 and 2015 over 6,000 women aged 18 or older were recruited in Hungary. Liquid-based cytology (LBC), high-risk HPV (hrHPV) DNA detection and single target host gene methylation test of the promoter sequence of the POU4F3 gene by quantitative methylation-specific polymerase chain reaction (PCR) were performed from the same liquid-based cytology sample. The current analysis is focused on the baseline cross-sectional clinical results of 5,384 LBC samples collected from subjects aged 25 years or older. The performance of the CONFIDENCE HPV™ test was found to be comparable to the cobas™ HPV test with good agreement. When applying the CONFIDENCE Marker™ test alone in hrHPV positives, it showed significantly higher sensitivity with matching specificity compared to LBC-based triage. For CIN3+ histological endpoint in the age group of 25–55 and 50–55, the methylation test of POU4F3 achieved relative sensitivities of 1.74 (95% CI: 1.25–2.33) and 1.64 (95% CI: 1.08–2.17), respectively, after verification bias adjustment. On the basis of our findings, POU4F3 methylation as a triage test of hrHPV positives appears to be a noteworthy method. We can reasonably assume that...
TRACE Study

Triage and Risk Assessment of Cervical Precancer by Epigenetic Biomarker

NEUMANN Kft. & Cellcall Kft., Budapest, Hungary
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Gábor Sobel MD, PhD

*until 2015
CONFIDENCE™ Assay

CONFIDENCE™

HPV + CONFIDENCE™

Marker

- Viral DNA test
- Multiplex RT-PCR
- hrHPV genotyping
  - HPV16 and HPV18
- Other hrHPV in group
  - 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- Validated on self collected samples

- Human epigenetic biomarker
- Quantitative MS-PCR on bisulphite converted DNA
- Hypermethylation of POU4F3 (POU Class 4 Homeobox 3) gene

NEUMANN
STUDY POPULATION, aged 18-65, n=6761

STUDY POPULATION, aged 25-65, n=5793

OUTPATIENT POPULATION
Enrolled subjects
aged 25-65, n=5705

ONCOLOGY CENTER POPULATION
Enrolled subjects
aged 25-65, n=88

Patient history, VIA, LBC sampling

LBC CITOLOGY &
diagnostic HPV

CONFIDENCE HPV™
CONFIDENCE Marker™

## Sample characteristics of the study sites

### Per protocol population aged 25-65

<table>
<thead>
<tr>
<th>SITE</th>
<th>FESZ</th>
<th>PROFILAKT</th>
<th>AMORA</th>
<th>SZSZBMK</th>
<th>ISTVAN</th>
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<tbody>
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<td>site</td>
<td>characteristics</td>
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<tr>
<td></td>
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<td>outpatient</td>
<td>outpatient</td>
<td>oncology center, capital, public</td>
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<td>private</td>
<td>private</td>
<td>country side,</td>
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<td>public</td>
<td></td>
</tr>
<tr>
<td>patient</td>
<td>management</td>
<td></td>
<td></td>
<td></td>
<td>treatment</td>
</tr>
<tr>
<td></td>
<td>screening and</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>follow-up</td>
<td></td>
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<tr>
<td>interventions</td>
<td>VIA, LBC sampling,</td>
<td>LBC cytology, hrHPV</td>
<td>biomarker test,</td>
<td>VIA, LBC sampling,</td>
<td>hrHPV detection, biomarker test, cone</td>
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<td></td>
<td>LBC sampling,</td>
<td>detection,</td>
<td>cone/punch biopsy</td>
<td>LBC sampling, LBC</td>
<td>biopsy, hysterectomy</td>
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<td>LBC cytology,</td>
<td>hrHPV detection,</td>
<td></td>
<td>cytology, hrHPV</td>
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<tr>
<td></td>
<td>hrHPV detection,</td>
<td>biomarker test,</td>
<td></td>
<td>detection,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>biomarker test,</td>
<td>cone biopsy,</td>
<td></td>
<td>cone biopsy,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cone biopsy,</td>
<td>hysterectomy</td>
<td></td>
<td>hysterectomy</td>
<td></td>
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<tr>
<td>total number</td>
<td>1225</td>
<td>437</td>
<td>1319</td>
<td>2333</td>
<td>70</td>
</tr>
<tr>
<td>hrHPV+ (rate)</td>
<td>294 (24%)</td>
<td>128 (29%)</td>
<td>306 (23%)</td>
<td>498 (21%)</td>
<td>61 (87%)</td>
</tr>
<tr>
<td>confirmed</td>
<td>25 (2%)</td>
<td>17 (3.9%)</td>
<td>45 (3.4%)</td>
<td>11 (0.5%)</td>
<td>69 (99%)</td>
</tr>
<tr>
<td>CIN2+</td>
<td>11</td>
<td>10</td>
<td>15</td>
<td>7</td>
<td>52</td>
</tr>
<tr>
<td>CIN2+ and</td>
<td>11</td>
<td>10</td>
<td>14</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>hrHPV+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>crude sensitivity* for CIN2+ in biomarker based triage of hrHPV+ cases</td>
<td>81.8 (48.2-97.7) [9/11]</td>
<td>100 (58.7-100) [10/10]</td>
<td>78.6 (49.2-95.3) [11/14]</td>
<td>83.3 (35.8-99.6) [5/6]</td>
<td>90.0 (78.1-96.7) [45/50]</td>
</tr>
<tr>
<td>crude sensitivity* for CIN2+ in LBC cytology based triage of hrHPV+ cases</td>
<td>100.0 (61.6-100.0) [11/11]</td>
<td>80.0 (44.4-97.5) [8/10]</td>
<td>85.7 (57.2-98.2) [12/14]</td>
<td>66.7 (22.3-95.7) [4/6]</td>
<td>76.0 (61.8-86.9) [38/50]</td>
</tr>
<tr>
<td>median M-index of CIN2+ cases (IQR)</td>
<td>158 (488)</td>
<td></td>
<td></td>
<td></td>
<td>185 (657)</td>
</tr>
</tbody>
</table>

Study design

EXCLUDED CASES (n=409)
- absent HPV result (n=189)
- invalid HPV result (n=7)
- absent citology result (n=31)
- invalid citology result (n=2)
- absent biomarker result (n=190)
- invalid biomarker result (n=100)
- invalid histology result (n=2)
- out-of-time range confirmation (n=43)

CASES WITH CONFIRMATION within 12 months, n=167
(from outpatient clinics n=95, from oncology center n=71)

PER PROTOCOL STUDY POPULATION
n=5,384, aged 25-65
• LBC cytology (ASCUS+/-)
• CONFIDENCE HPV™ (hrHPV pos/neg)
• CONFIDENCE Marker™ (MET+/-)

hrHPV positives
n=1,287

ASCUS+ MET+
n=159 → n=88
   → n=71
      → n=5
         n=66

ASCUS+ MET-
n=205 → n=179
   → n=26
      → n=19
         n=7

ASCUS- MET+
n=296 → n=272
   → n=24
      → n=10
         n=14

ASCUS- MET-
n=627 → n=604
   → n=23
      → n=19
         n=4

Confirmed cases
N=144

hrHPV negatives
n=4,097

CIN2+ cases
N=91

Number of cases identified by the test results among hrHPV positives

Number of cases identified by the test results among hrHPV positives

Number of cases identified by the test results among hrHPV positives

Number of cases identified by the test results among hrHPV positives

Number of cases identified by the test results among hrHPV positives

Methylation vs. Cytology

**CONFIDENCE™ HPV**

Good agreement compared to cobas® HPV test

<table>
<thead>
<tr>
<th>Overall agreement</th>
<th>92.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive agreement</td>
<td>Negative agreement</td>
</tr>
<tr>
<td>PPA</td>
<td>82.6% (95% CI: 80.5-84.7)</td>
</tr>
<tr>
<td>cPPA</td>
<td>70.4%</td>
</tr>
<tr>
<td>SE to REF</td>
<td>87.2%</td>
</tr>
</tbody>
</table>

Cohen’s kappa 0.77 (95% CI: 0.74-0.81)

PABAK 0.84 (95% CI: 0.82-0.86)

‘HC2, cobas, CLART and APTIMA …’

‘42 to 58% positive agreement on any compared pair of assays’

‘0.53-0.75 kappa value’ (HORIZON Study, Rebolj et al, 2014)

High clinical equivalency for CIN2+ and CIN3+

n=3270, aged 25-65

<table>
<thead>
<tr>
<th>25-65, CIN2+</th>
<th>CONFIDENCE™ HPV</th>
<th>cobas® HPV</th>
<th>Relative ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SE</strong></td>
<td>95.2 (88.1-98.7)</td>
<td>96.4 (89.8-99.3)</td>
<td>0.99 (0.93-1.05)</td>
</tr>
<tr>
<td><strong>SP</strong></td>
<td>77.8 (76.2-79.2)</td>
<td>79.9 (78.5-81.4)</td>
<td>0.97 (0.95-0.99)</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>10.4 (8.2-12.8)</td>
<td>11.5 (9.2-14.1)</td>
<td>0.90 (0.67-1.21)</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>99.8 (99.6-99.9)</td>
<td>99.8 (99.6-99.9)</td>
<td>0.99 (0.99-1.01)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>25-65, CIN3+</th>
<th>CONFIDENCE™ HPV</th>
<th>cobas® HPV</th>
<th>Relative ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SE</strong></td>
<td>98.5 (91.8-99.9)</td>
<td>98.5 (91.8-99.9)</td>
<td>1.00 (0.96-1.04)</td>
</tr>
<tr>
<td><strong>SP</strong></td>
<td>77.4 (75.9-78.9)</td>
<td>79.6 (78.1-81.4)</td>
<td>0.97 (0.95-0.99)</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>8.5 (6.7-10.8)</td>
<td>9.4 (7.3-11.8)</td>
<td>0.91 (0.66-1.27)</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>99.9 (99.8-99.9)</td>
<td>99.9 (99.8-99.9)</td>
<td>0.99 (0.99-1.01)</td>
</tr>
</tbody>
</table>

High discriminative power

Methylation level of the target gene is independent from hrHPV positivity and elevated in high risk lesions.

CONFIDENCE™ Marker

High discriminative power

Methylation level of the target gene is independent from hrHPV positivity and elevated in high risk lesions.

ROC analysis - Age dependent cut-off

## Performance in hrHPV+ triage

CIN2+ endpoint, study population aged 25-65 (total n=5,384; hrHPV+ n=1.287)

<table>
<thead>
<tr>
<th></th>
<th>POU4F3 marker</th>
<th>LBC cytology</th>
<th>Relative ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crude</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>88.2 (85.5-91.0)</td>
<td>80.1 (71.6-88.2)</td>
<td>1.10 (1.00-1.24)</td>
</tr>
<tr>
<td>SP</td>
<td>72.9 (50.8-89.5)</td>
<td>54.7 (41.4-68.3)</td>
<td>1.36 (0.86-1.95)</td>
</tr>
<tr>
<td><strong>Adjusted</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>70.1 (55.5-84.5)</td>
<td>42.7 (33.0-56.3)</td>
<td>1.67 (1.23-2.24)</td>
</tr>
<tr>
<td>SP</td>
<td>81.4 (58.0-95.4)</td>
<td>80.4 (75.1-85.5)</td>
<td>1.01 (0.70-1.22)</td>
</tr>
</tbody>
</table>

CONFIDENCE™ assay can provide **significantly higher sensitivity** than cytology based triage with comparable specificity.

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## Performance in hrHPV+ triage

CIN3+ endpoint, study population aged 25-65 (total n=5,384; hrHPV+ n=1.287)

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<thead>
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<th>POU4F3 marker</th>
<th>LBC cytology</th>
<th>Relative ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crude</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>89.6 (84.6-94.6)</td>
<td>80.1 (70.9-88.7)</td>
<td>1.12 (0.99-1.27)</td>
</tr>
<tr>
<td>SP</td>
<td>60.9 (41.0-77.9)</td>
<td>47.4 (36.1-59.3)</td>
<td>1.31 (0.82-1.87)</td>
</tr>
<tr>
<td><strong>Adjusted</strong></td>
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<td></td>
</tr>
<tr>
<td>SE</td>
<td>72.3 (58.5-86.8)</td>
<td>42.3 (31.2-55.7)</td>
<td>1.74 (1.25-2.33)</td>
</tr>
<tr>
<td>SP</td>
<td>76.5 (52.1-91.9)</td>
<td>77.8 (73.0-82.9)</td>
<td>0.98 (0.66-1.21)</td>
</tr>
</tbody>
</table>

CONFIDENCE™ assay can provide **significantly higher sensitivity** than cytology based triage with comparable specificity.
Using more markers in hrHPV+ triage

CIN2+ endpoint, study population aged 25-65 (total n=5,384; hrHPV+ n=1.287)

<table>
<thead>
<tr>
<th></th>
<th>POU4F3 marker with 16/18 typing</th>
<th>LBC cytology with 16/18 typing</th>
<th>Relative ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude SE</td>
<td>92.3 (86.3-97.5)</td>
<td>93.3 (87.4-98.0)</td>
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<tr>
<td></td>
<td>Crude SP</td>
<td>43.2 (30.4-57.4)</td>
<td>28.2 (16.3-40.4)</td>
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<tr>
<td></td>
<td>Adjusted SE</td>
<td>82.3 (69.9-95.1)</td>
<td>75.3 (62.2-89.7)</td>
</tr>
<tr>
<td></td>
<td>Adjusted SP</td>
<td>67.5 (58.0-76.8)</td>
<td>65.0 (56.5-72.2)</td>
</tr>
</tbody>
</table>

Applying more tests parallel in hrHPV+ triage resulted in higher specificity but decreased specificity

Screening with High Confidence

We recommend primary hrHPV screening with highly sensitive but specific biomarker triage.

CONFIDENCE™ HPV
hrHPV DNA test with genotyping HPV16 and 18

CONFIDENCE™ Marker
host gene methylation test of single target gene