CINtec PLUS Immunocytochemistry
Effective Triage Tool for Cervical Cancer Screening
3rd NordiQC Conference on Applied Immunohistochemistry, Aalborg, 6th June 2017
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Abnormal cytology can’t hide anymore
Cervical Cancer
Statistics and Facts

- **3rd most common cancer** in women worldwide; mortality (women): 4th
- Most common cause of cancer death in the world where Pap tests are not available
- **530,000 new cases/year**, mostly in developing countries
- Approximately 270,000 patients (51%) **will die from it**

- **Up to 1/3** of Cervical Cancers occur in screened women **with a normal Pap**
- **Almost 100% preventable/curable**
  - if detected early with proper screening, management and treatment

Cervical Cancer

Human Papilloma Virus (HPV)

- Sexually transmitted infection
  - Higher prevalence in younger women
    - ~25% of women under 30
    - ~10% of women over 30

- 2 Sub-Types:
  - **Low Risk** (LR-HPV) typically cause genital warts (condyloma aka) but may be associated with pre-cancerous cervical lesions as well
  - **High Risk**: 14 genotypes (HR + VHR-HPV) cause >99% of cervical cancers
    - 2 genotypes: 16 and 18 alone cause ~70% of cervical cancer

- Most HPV infections **clear** in months but some **persist** for years; persistent HPV infections can lead to cervical cancer

- Only 5% of HPV infected patients (high-risk HPV types) develop cervical cancer
HPV and Development of Cervical Cancer

Natural History of HPV Infections and Cervical Disease

- Cx cancer is preceded by precancerous lesions that progress w/o symptoms over years

mod. nach: Trunk et al., Der Pathologe 2005, 26:283
Cervical Cancer Screening Algorithm
Example Cytology and Reflex HPV Test for ASC-US

- Screening with Pap Tests has improved disease morbidity/mortality
  - Mortality has been decreased by 70%

Pap and HPV testing have been implemented in routine screening to identify those women most likely to harbor high-grade disease.
Cervical Cancer Screening by Cytology

Drastically reduced Cervical Cancer Incidence... but Efforts now plateau

- Incidence levelled off – cytology not reducing cervical cancer incidence further
  
- In recent audits of both Kaiser Permanente and in Sweden, 32% and 24%, respectively, of women with invasive cervical cancer are attributable to Pap test detection failure

![Graph showing age-standardized rate per 100,000 women over years (1975, 1985, 1995, 2005) for Denmark, Finland, Norway, and Sweden.](image)

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2 Leyden et al. JNCI 2005; 97: 675
3 Andrae et al. JNCI 2008; 100: 622
Cervical Cancer Screening
Common Algorithms show Significant Limitations

- Specificity is good
- Sensitivity as a single test is unsatisfactory

- Missed high-grade disease
- Unnecessary diagnostic follow-up procedures and overtreatment
- Resource intensity, unnecessary costs
**HPV Screening Superior across multiple Studies**

*The Superiority of HPV Sensitivity over Pap has been consistently repeated, including ATHENA (cobas HPV test)*

**Data and Others**

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity* for ≥CIN2 (%)</th>
<th>PAP</th>
<th>HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bigras (n=13,842)</td>
<td>58.7</td>
<td>97.0</td>
<td></td>
</tr>
<tr>
<td>Cardenas (n=1,850)</td>
<td>44.0</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Coste (n=3,080)</td>
<td>65.0</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Kulasingam (n=774)</td>
<td>38.3</td>
<td>62.7</td>
<td></td>
</tr>
<tr>
<td>Mayrand (n=9,977)</td>
<td>56.4</td>
<td>97.4</td>
<td></td>
</tr>
<tr>
<td>Petry (n=7,908)</td>
<td>43.5</td>
<td>97.8</td>
<td></td>
</tr>
</tbody>
</table>

Average increase: 35.7%


*Sensitivity as a percentage for ≥CIN2 (carcinoma in situ of the cervix).*
Cervical Cancer Screening Algorithm
Current US FDA approved Algorithm for Primary HPV Screening

- Routine screening
  - HPV−
    - 12 other HPV+
      - HPV16/18+
        - COLPOSCOPY
  - Cytology
    - NILM
      - Follow up in 12 months
    - ≥ ASC-US
      - COLPOSCOPY
Improving Cervical Cancer Screening
Opportunity for HPV Genotyping and Biomarkers

- Pap cytology has unsatisfactory sensitivity, with good specificity
- HPV testing provides best sensitivity, but lowers specificity
- Need for algorithms that increase sensitivity without lowering specificity

New options for improved screening and patient management strategies:
- HPV 16/18 genotyping
- p16/Ki-67 dual-stained cytology
CINtec PLUS Cytology
Adding Biomarker Information to Cervical Cytology

Combination of p16 and Ki-67 biomarker in a single ICC test

• p16/Ki-67 Cocktail & Dual Color Detection in one cytology-optimized ICC kit
  – Fully automated and manual kit available
  – Standardised staining protocols

• Brown nuclear/cytoplasmic p16 & Red nuclear Ki-67 stain

• Applicable on conventional smears and liquid based cytology samples

• Sensitivity and specificity data supported by significant trials
p16 Biomarker

Overview

- Cellular protein involved in cell-cycle control
  - restricted to the G0 Phase

- Over-expression of p16 can be used as a biomarker for pre-cancerous and cancerous cervical lesions
  - Surrogate for inactivation of the tumor suppressor protein pRB by high-risk HPV E7 oncoproteins
  - Direct link between over-expression of p16 and pathogenetic process of cervical dysplasia
Proliferation Marker Ki-67

Overview

- Ki-67 protein can be detected within the nucleus of normal proliferating cells

- Expression restricted to the G1-, S-, G2 and M-phase of the cell cycle
  - Marker of cell proliferation
  - No expression in non-dividing cells
  - Absent in G0-phase of the cell cycle

Proliferating cells in normal squamous cervical epithelium show nuclear Ki-67 staining
Simultaneous expression of p16 and Ki-67 is mutually exclusive of each other in cells with intact cell cycle control

p16/Ki-67 dual staining in the same cell indicates cell cycle deregulation

Identification of double-immunoreactive cells in cervical cytology preparations can be an indicator for the presence of high-grade cervical dysplastic lesions

p16: Cell-cycle arrest

Ki-67: Cell-cycle progression

p16/Ki-67 Co-expression
Interpretation of p16/Ki-67 Dual-Staining Principle

Negative Result

• Cervical epithelial cells staining with
  – Blue counterstain only
  – p16, cytoplasm and/or nuclei only
  – Ki-67, nuclei only

Positive Result

• Detection of at least one p16 / Ki-67 dual-stained cervical epithelial cell
  – p16 cytoplasmic staining and Ki-67 nuclear staining
  – Isolated cell(s) or within cell cluster
  – Special algorithm for cluster evaluation must be followed
Interpretation of p16/Ki-67 Dual-Staining

Examples of Staining Results

negative  positive
How to make Cervical Cancer Screening and Management more effective?

**Screening**
- Higher sensitivity as compared to Pap cytology
- Less frequent testing based on high NPV of single screening result

**Patient Management**
- Provide optimal triage tool
- Minimize number of false-positive screening results
CINtec PLUS Dual-Staining for Cytology
Data to Support Use in Several Settings

• Triage method of ASC-US
  - especially younger women or HPV 16/18 (-)

• Triage method of LSIL
  - first real option for triage

• Triage method of NILM / hrHPV (+)
  - likely in conjunction with genotyping for HPV 16/18

• Triage method of hrHPV (+)
  - when HPV testing is the primary screening and likely with genotyping

CINtec PLUS Dual-Staining for Cytology
Trials to validate Clinical Utility in ASC-US / LSIL

- **PALMS Trial:** prospective screening trial in more than 27,000 women across Europe
- **EEMAPS:** retrospective ASC-US / LSIL triage study
- **Uijterwaal et al.:** cross-sectional and longitudinal performance of p16/Ki-67 dual-stain cytology as a triage for BMD compared with HPV test results
- **Waldstrom et al.:** comparison APTIMA mRNA HPV assay with p16/Ki-67 in women with LSIL
- **Wentzensen et al.:** colposcopy clinic cohort study
- **Killeen et al.:** prospective colposcopy clinic cohort
Clinical Validation Data, ASC-US Triage

* Dual Stain versus HPV for CIN2+

**Sensitivity**

- **PALMS**
  - Dual Stain: 94%
  - HPV: 100%
- **EEMAPS**
  - Dual Stain: 92%
  - HPV: 91%
- **Wentzensen**
  - Dual Stain: 82%
  - HPV: 97%
- **Killeen**
  - Dual Stain: 94%
  - HPV: 91%

**Specificity**

- **PALMS**
  - Dual Stain: 78%
  - HPV: 61%
- **EEMAPS**
  - Dual Stain: 81%
  - HPV: 36%
- **Wentzensen**
  - Dual Stain: 82%
  - HPV: 62%
- **Killeen**
  - Dual Stain: 62%
  - HPV: 15%

Clinical Validation Data, LSIL Triage

*Dual Stain versus HPV for CIN2*+*

**Sensitivity**
- PALMS: 86% Dual Stain, 98% HPV
- EEMAPS: 94% Dual Stain, 96% HPV
- Wentzensen: 87% Dual Stain, 92% HPV
- Uijterwaal*: 90% Dual Stain, 97% HPV
- Waldström: 89% Dual Stain, 92% HPV

**Specificity**
- PALMS: 54% Dual Stain, 19% HPV
- EEMAPS: 68% Dual Stain, 19% HPV
- Wentzensen: 58% Dual Stain, 35% HPV
- Uijterwaal*: 73% Dual Stain, 68% HPV
- Waldström: 51% Dual Stain, 36% HPV

* Triage data for Borderline/mild dyskaryosis (BMD)

Oklahoma Study of Women with Colposcopy Referral *Wentzensen et al. 2012 (NCI)*

- Women referred to colposcopy in Oklahoma for evaluation of abnormal Pap cytology results (n=625)
- Repeat cytology, hrHPV testing, HPV genotyping, and CINtec PLUS for all women
- Biopsy confirmed CIN2+/CIN3+ as clinical endpoints
- **LSIL Triage: May reduce number of colposcopies up to 50%**

<table>
<thead>
<tr>
<th></th>
<th>Dual stain</th>
<th>hrHPV (+)</th>
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</thead>
<tbody>
<tr>
<td>ASC-US</td>
<td>40%</td>
<td>72%</td>
</tr>
<tr>
<td>LSIL</td>
<td>69%</td>
<td>85%</td>
</tr>
<tr>
<td>ASC-H</td>
<td>83%</td>
<td>90%</td>
</tr>
<tr>
<td>HSIL</td>
<td>95%</td>
<td>95%</td>
</tr>
</tbody>
</table>

CINtec PLUS Dual-Staining for Cytology
Trials to validate Clinical Utility in HPV (+)

- **Petry et al:** retrospective, Wolfsburg study of 425 women

- **Wentzensen et al:** prospective, NCI-Kaiser Permanente cohort of 1,509 HPV (+) women

- **PALMS:** prospective, screening trial in more than 27,000 women across Europe

- **ATHENA & p16/Ki-67 Triage:** retrospective, ATHENA study sub-population of women 25 or older with cobas HPV positive result, end-point biopsy CIN2+
  - [http://www.gynecologicconcology-online.net/article/S0090-8258(16)31508-6/pdf](http://www.gynecologicconcology-online.net/article/S0090-8258(16)31508-6/pdf)
Women aged 30 or older and participating in Pap / HPV co-testing program
- LBC (ThinPrep) Pap cytology and hc2 HPV testing
- Women positive on either test were referred to colposcopy

p16 / Ki-67 dual-staining retrospectively performed on residual LBC vial (at baseline) in Pap neg/HPV positive women

Triage of Pap neg/HPV pos Women ≥30 y

**Wolfsburg Study**

- Pap neg, HPV pos
  - Dual Stain pos: n=108
    - <CIN2: n=74, 69%
    - CIN2+: n=34, 31%
  - Dual Stain neg: n=317
    - <CIN2: n=314, 99%
    - CIN2+: n=3, 1%

Petty et al. Gynecol Oncol. 2011;121:505-9
Triaging HPV-positive women with p16/Ki-67 dual-stained cytology: Results from a sub-study nested into the ATHENA trial

Thomas C. Wright Jr. a,*, Catherine M. Behrens b, James Ranger-Moore c, Susanne Rehm c,d, Abha Sharma b, Mark H. Stoler e, Ruediger Ridder c,d

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d Roche mtm laboratories AG, Mannheim, Germany
e University of Virginia, Charlottesville, VA, USA
ATHENA & p16/Ki-67 Dual Stain Triage
Shows optimized Screening Strategy

**cobas HPV & CINtec PLUS**

- Sensitivity: 46.5 vs 74.3
- Specificity: 89.9 vs 82.5
- Increase in sensitivity: 59.8%

**Study Design**

- Retrospective study; end-point biopsy CIN2+
- ATHENA study sub-population of women 25 or older with cobas HPV positive result
- Comparison of HPV primary screening with LBC triage vs HPV primary screening with 16/18 genotyping and CINtec PLUS triage for 12 other hrHPV
- Testing performed on residual ATHENA samples in PreservCyt vials

Source: http://www.gynecologicconcolorology-online.net/article/S0090-8258(16)31508-6/pdf
Better Outcomes at Lower Cost

HECON Model: cobas HPV & CINtec PLUS vs Pap

Clinical Impact

Cervical Cancer Detection Rate

- **Pap with HPV triage**: 51.8%
- **cobas HPV with CINtec PLUS triage**: 90.0%
  - +38%

Total Cost Per Screened Patient (€)

- **Pap with HPV triage**: 10,30
- **cobas HPV with CINtec PLUS triage**: 9,50
  - -8%

Source: Petry et al., IPV abstract 2015

Annual Payer savings: €10,348,541
p16/Ki-67 Biomarker-Combination for Cervical Cytology

- Indicates cell cycle deregulation caused by oncogenic transformed hrHPV infections
- Significant increase in sensitivity compared to Pap cytology
- Improved specificity compared to HPV testing

Effective Triage Tool

- Enables medical decision in women being hrHPV pos but not 16/18:
  - Finds diseases while drastically limiting number of referrals
  - Decreased overtreatment, decreased number of colposcopies
Doing now what patients need next

Thank you for your attention!
Doing now what patients need next