CINtec PLUS - Effective Biomarker Combination for Cervical Cancer Screening

29th May 2015, Clinical Hospital Centre Rijeka, M. Wolff, Roche Diagnostics
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
- Almost 100% preventable/curable if detected early
- Screening with Pap Tests has improved disease morbidity/mortality
  - Mortality has been decreased by 70 %
  - Technology has not changed in the past 50 years
  - Pap and HPV testing have been implemented in routine screening/triage testing to identify those women most likely to harbor high-grade disease

Purpose: to identify women at greatest likelihood for cervical disease to send for diagnosis
Cervical Cancer

Human Papilloma Virus

- 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 (aka condyloma) 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
General Principles of Cervical Cancer Screening

Two important pieces of clinical information desired

1. Which women are at risk for developing cervical cancer?
   - Negative Predictive Value (NPV); **Rule out**
   - Three levels of Risk based on HR-HPV Status:
     - HR-HPV Negative: Low/None
     - HR-HPV Panel Positive: Moderate
     - Type 16/18 Positive: Highest
   - Drives the optimal screening intervals

2. Which women are most likely to have established high-grade disease and therefore most likely to develop cervical cancer?
   - Positive Predictive Value (PPV); **Rule in**
   - Drives the intervention protocol
p16 Biomarker

Overview

- Cellular protein involved in cell-cycle control
- 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
- Measuring the carcinogenic activity of HPV at the cellular level
  - Independent of morphology and individual observer
  - Patient age independent
  - HR-HPV type independent
Healthy squamous basal and parabasal cells spend most of their time in cell cycle arrest.

Mitosis is partly regulated by the protein complex of retinoblastoma (pRB) and E2F.
- When bound to each other, they keep the cell at rest.

If needed, hormones will signal the cells to divide.

pRB de-couples from E2F resulting in cell cycle progression and mitosis.

Following mitosis, the cell expresses low levels of p16 (quantities too low to be detectable by IHC); this facilitates the re-binding of pRB to E2F putting the cell cycle back into arrest.
Transient HPV Infection

*Virus does not interfere with normal cell cycle control*

- Upon infection with HPV, viral DNA is introduced into basal cells.
- The virus replicates in the differentiated cell layers and new infectious particles are produced.
- Transient HPV infections do not affect the mechanism of cell cycle control.
Transforming HPV Infection

*Viral oncoprotein E7 interferes with cell cycle control*

- If the HPV infection does not clear and becomes **persistent**, it can begin producing viral oncoprotein E7 (typically HR-HPV genotypes)

- E7 interferes with the cell cycle, causing the cells to continuously divide and create pre-cancerous lesions; if untreated, it can become cancer

- E7 impairs the function of pRB, disrupting its ability to bind to transcription factor E2F marking the onset of the oncogenic transforming process. This leads to:
  1. Deregulated cell proliferation
  2. p16 over-expression, which is detectable by IHC
Proliferations 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

Normal cervical squamous epithelium
P16 expression in normal cells

Cell cycle arrest
p16, Ki-67

**P16 expression in normal cells**

- **Cell cycle arrest**

**Ki-67 expression in normal cells**

- **Cell cycle progression/proliferation**
p16, Ki-67, Co-Expression

P16 expression in normal cells

Ki-67 expression in normal cells

Simultaneous co-expression

Cell cycle arrest

Cell cycle progression/proliferation

Cell cycle deregulation
p16 & Ki-67
Biomarker-Combination

- 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
CINtec PLUS Products
Unique Solutions for Cytology

• Manual kit and fully automated solution on BenchMark GX, XT and ULTRA series

• Can be applied on Conventional or LBC slides

• 3 – 3,5 hours walk away staining time (manual: 5,5 – 6 hours hands on time)
Interpretation of p16/Ki-67 Dual Staining

**Principle**

Locator function: Ki-67

Interpreter function: p16

Screen slide for p16/Ki-67 Dual-stained cells

At least one Dual-stained cell present?

- **Yes**
- **No**

**Positive**

**Negative**
Interpretation of p16/Ki-67 Dual Staining

Definition of a dual-stained cell

- The cytoplasm is stained brown and the nucleus stained red
- p16 signal (brown) and Ki-67 signal (red) **within the same cell**
  - Any level of expression, weak or strong
- The red stained nucleus and the brown stained cytoplasm must be **within the same plane of focus** (level)
- Interpretation is independent from morphologic criteria
- One dual-stained cell indicates a positive test result
Single and dual-stained cells

Examples from the same case

Single p16  Single Ki-67  Dual-stained cell
Only p16-stained cells
Only Ki-67 stained cells
Single dual-stained cells
Interpretation of p16/Ki-67 Dual Staining

Recommended screening procedure

• Systematic overlapping fields of view

• Initially search for isolated dual-stained cells
  - Easiest to evaluate

• If an isolated dual-stained cell has been identified, the stained nucleus and cytoplasm must be clearly allocated to the same cell
  - Verify the cytoplasm and nucleus are in the same plane of focus (no overlying or underlying red nucleus)

• When evaluating cell clusters with red and brown staining, an interpretation algorithm must be followed
Interpretation algorithm for cell clusters
Interpretation of red and brown stained cell clusters

1. **Look for dual-stained cells at the edge of the cluster**
   - If yes → test result is positive, dual-stained cells present
   - If no → proceed with step 2

2. **Check if the p16 staining of the cluster can be defined as diffuse or focal**
   - Focal p16 staining: no further evaluation of this negative cluster
   - Diffuse p16 staining: proceed with step 3

3. **Check if the p16 staining is specific to the cytoplasm**
   - p16 staining is not specific (mucus or non-specific background staining) → no further evaluation, a negative cluster
   - Specific p16 staining: proceed with step 4

4. **Are the Ki-67 staining, red nuclei over/underlying or embedded?**
   *(use the fine focus knob)*
   - Ki-67 staining, red nuclei are over/underlying → no further evaluation, a negative cluster
   - Ki-67 staining, red nuclei are embedded → test result is positive – dual-stained cells present
Cluster with dual-stained cells
One isolated dual-stained cell at the edge
No dual stain in individual cells
Cluster with no dual-stained cells
Non-specific background staining

Detection chemistry chromogen can become trapped within cells, mucus or other cellular debris and could cause:

- Various intensities: low/medium/high
- Various distribution: few cells to all cells
- Homogeneous/heterogeneous
Specific staining

*Ki-67 (red) only*

- Proliferating cells show only ki-67 signal (red)
  - without background
  - without specific p16 signal
  - negative test result
Non-specific brown background staining

Be aware to avoid false positive results

Proliferating cells (Ki-67 positive) in combination with non-specific background staining could cause a false positive result.
Interpretation with non-specific brown background

Key recommendation for interpretation

The brown p16 stain must be more intense than the non-specific surrounding brown background to evaluate the cell(s) as positive for p16 & Ki-67.

Do not evaluate cells with non-specific brown background, regardless of the Ki-67 staining.
Dual-stained cell with non-specific background

Example

Positive cell for CINtec PLUS Cytology:

The brown p16 stain in the cytoplasm of this cell with a red Ki-67 stained nucleus is more intense than the non-specific brown surrounding background.

Non-specific brown background staining in reference cell.
Dual-stained cells, low level expression

*Example*

Must always compare the p16 (brown) staining to your reference cells
- Both negative or cells with non-specific brown background staining
Neutrophils staining red

Artifact
**Cornflaking**

*Drying artifact*

Air drying that occurs before applying aqueous mounting media
Fading of the fast red stain
Artifact from improper post processing procedure (mounting/coverslipping)

- Any contact with alcohol leads to Fading of Fast Red chromogen
- Aqueous mounting procedure to prevent fading
- The use Roche Hematoxylin, do not use alcoholic Hematoxylin

Cause: contact with alcohol during mounting/coverslipping procedure or counterstaining
Aqueous mounting procedure

Cracking Artifact

Cracks: A result of incomplete drying of aqueous mounting media
p16 / Ki-67 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
- **Wentzensen et al**: Colposcopy clinic cohort
- **Petry et al**: Screening for Cervical Cancer Precursors With p16/Ki-67 Dual-Stained Cytology: Results of the PALMS Study
- **Waldstrom et al**: comparison APTIMA mRNA HPV assay with p16/Ki-67 in women with LSIL
- **Ujeterwall et al**: CINtec PLUS Cytology vs. HC2 in biopsy-confirmed CIN 3
CINtec PLUS Data Summary

High Sensitivity PLUS High Specificity for CIN2+
Three significant clinical trials

Pan-European trials demonstrate strong clinical data

| PALMS | • Primary screening, ASC-US, LSIL Marker Study
|       | • Multinational, multicenter, prospective diagnostic study in > 27,000 women |
| EEMAPS| • European Equivocal or Mildly Abnormal Pap Cytology Study
|       | • Over 200 cases of biopsy confirmed high-grade disease from a total of 777 ASC-US and LSIL cases (Schmidt et al., Cancer Cytopathol 2011) |
| Wolfsburg | • The Wolfsburg Pap/HPV Co-Testing Study
|       | • Over 4,400 women aged 30 and above (Petry et al., Gynecol Oncol 2011) |

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<thead>
<tr>
<th>CINtec® PLUS</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Screening</td>
<td>90 – 93%</td>
<td>95 – 97.5%</td>
</tr>
<tr>
<td>LSIL Triage</td>
<td>85 – 94%</td>
<td>54 – 68%</td>
</tr>
<tr>
<td>ASCUS Triage</td>
<td>92 – 94%</td>
<td>78 – 81%</td>
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<tr>
<td>Pap neg / HR-HPV pos Triage</td>
<td>92%</td>
<td>85%</td>
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CINtec PLUS Cytology data: Wentzensen (NCI)
CINtec PLUS Cytology positivity rate in Colposcopy Referral

**Wentzensen et al; Clinical Cancer Research 2012**

CINtec PLUS Cytology in colposcopy referral
- 625 women referred to colposcopy following abnormal cytology result
- All women had repeat cytology, hrHPV testing, HPV genotyping, and CINtec PLUS
- LSIL Triage: May reduce number of colposcopies up to 50%

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<thead>
<tr>
<th></th>
<th>Dual stain</th>
<th>hrHPV (+)</th>
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<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>
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Wentzensen et al. Clinical Cancer Research 2012
# Clinical Utility of CINtec PLUS

## Summary of study results

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<tr>
<th>Target</th>
<th>Claim</th>
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<tbody>
<tr>
<td>Screening</td>
<td>Significantly higher sensitivity than Pap with equal specificity</td>
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<tr>
<td>ASC-US (12GT hrHPV+)/LSIL</td>
<td>First technology that can efficiently triage women vs. repeat testing or direct colposcopy</td>
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<tr>
<td>Triage of HPV positive</td>
<td>First technology to effectively and immediately identify those women for direct to colposcopy</td>
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CINtec Histology
Unique and Fully Automated Solutions for Histology

Detects the over-expression of p16 in cervical biopsies

- Used in conjunction with H&E
- Highly sensitive and specific for identifying existing disease/lesions with oncogenic potential
- Interpretation of positive results is highly reproducible
- Data supported in 100+ publications and 2 large registration trials
- Included in ASCCP/CAP and WHO guidances
- Manual kit and fully automated solution on BenchMark series available
One Simple Path to the Decision to Biopsy
Providing a Safe Rule out Option

Clinical Initiation
- OBGYN visit

Screening algorithms
- HPV HR + → CINtec PLUS
- HPV 16/18 -> Reflex colposcopy

Management
- Pos GT 16/18
- Pos CINtec PLUS

Biopsy
- Colposcopy
- Treatment

Routine Screening
- HPV neg.
- HPV 16/18
- Other 12 HR
Doing now what patients need next