Utilization of the Biomarkers to Improve Cervical Cancer Screening

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• **Cervical cancer** is at the second most common cancer in women worldwide.

• **In Romania** is the highest incidence and mortality rates from Europe.
Screening for cervical cancer

- The single Pap smear test has limited sensitivity and specificity.

Limitations of Pap smear screening

- For a high grade lesion, the sensitivity of a single pap smear is only 60–80%
- Errors in sampling, slide preparation and interpretation are inherent in cytology
- Sampling for atypical glandular cells is exceptionally difficult
- False-positive rates range from 15–50%
- False-negative rates may reach 30%
Screening for cervical cancer

- Combined Pap smear with Colposcopy


Screening for cervical cancer

Combined HPV and Pap Testing
Better Predict Risk of Cervical Cancer


• **Human papillomavirus (HPV)** has been found to be associated with several types of cancer: cervical, vulvar, vaginal, penile, anal, and oropharyngeal.

Grulich AE, Jin F, Conway EL, Stein AN, Hocking J, Cancers attributable to human papillomavirus infection. Sex Health. 2010 Sep;7(3):244-52
• Certain types of HPV can produce local cellular changes of the cervix uteri epithelium. Pathologists agree that HPV infection is necessary for the development of precancerous cervical disease that may eventually progress to cervical cancer if left untreated.

• Over 100 different HPV types have been identified so far. Based on the risk of causing cervical cancer, they are grouped into low-risk and high-risk categories.

Polymerase Chain Reaction (PCR) determine:

- **HR-HPV (High-risk HPV):** 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68;
- **LR-HPV (Low-risk HPV):** 6, 11, 42;
- **Other HPV:** 26, 40, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108.

- **High-risk HPV DNA** is found in almost all cervical cancers (>99.7%), with **HPV16** being the most prevalent type in both low-grade disease and cervical neoplasia.

• Productive **infection by high-risk HPV types** is manifest as cervical flat warts or condyloma that shed infectious virions from their surface.

• Viral genomes are maintained as episomes in the basal layer, with viral gene expression being tightly controlled as the infected cells move towards the epithelial surface.

• **Cervical cancer** is induced by persistent infections with oncogenic human papilloma viruses, but cervical cancer usually does not develop unless high risk HPV infection persists over many years.

• While HPV infection is an indispensable factor, it is not sufficient to cause cancer.

• There are other contributing factors to the development of cancers such as smoking, immunosupression, etc.

• The **majority of acute HPV** infections induce low grade precursor lesions that are cleared spontaneously after several months in more than 90% of cases, and less than 10% eventually progress to high grade lesions or invasive cancer.

• Progression is characterized by the **deregulated expression of the viral oncogenes E6 and E7** in infected basal and parabasal cells.
Cervical cancer progression model

- Transitions are not well understood
- Histologic categories do not fully translate to functional progression model
- Majority of CIN2s and CIN3s do not progress to cancer
Novel biomarkers

- allow monitoring the essential molecular events in histological or cytological specimens
- are likely to improve the detection of lesions that have a high risk of progression in both primary screening and triage settings.

Biomarkers for cervical cancer screening

- Chrom. Instability: 3q, 5p
- Proliferation: Mcm2, Top2a, ki67
- p16
- Host and viral methylation
- HPV integration
- HPV oncogene mRNA

HPV DNA

Normal

HPV Infected

Progression

Precancer

Invasion

Cancer

Infection

Clearance

Regression

Wentzensen 2007 Dis Markers
Cuschieri and Wentzensen 2008 CEBP
Currently, the most frequently used biomarker in cervical cancer screening is the detection of HPV DNA.

For women over 30 years of age, testing for the presence of DNA from high-risk HPV types has been accepted as an adjunct to ASC-US cytology in primary screening. Women testing negative for HPV are considered at very low risk of developing cervical cancer and will be returned to routine screening.

Women testing positive for HPV, however, are considered at higher risk of harboring cervical dysplasia or cancer and are generally referred for colposcopic examination.

• **HPV DNA testing** has a very **high sensitivity** for the detection of high grade cervical disease, it has a very **low specificity** and PPV, unnecessarily raising patient anxiety levels and referrals to invasive colposcopic procedures.

• The HPV DNA story, therefore, clearly demonstrates that there is a need for **additional biomarkers** to discriminate lesions with a high risk of progression from those that will spontaneously regress.

=> HPV mRNA

The **intracellular targets for HPVs** include a number of regulatory proteins such as:

- cyclins
- cyclin dependent kinases
- cyclin inhibitors and
- cell cycle-associated proteins.

The **HPV E6 and E7 oncoproteins** inactivate the p53 and retinoblastoma protein tumor suppressors, respectively, leading to hyperproliferation and genomic instability.

• When the immune system is unable to clear an HPV infection, or the cervical cells are coping with a continual re-infection, an increased risk of cervical neoplasia exists.

• During persistent infection with HPV, the continued expression of two HPV oncoproteins, referred to as E6 and E7, alter the integrity of the cell cycle and disrupt the controlled processes of DNA repair, replication, and subsequent cell division.

• The detection of E6/E7 mRNA of high risk HPV genotypes may be a useful biomarker for revealing underlying cell abnormalities at greatest risk for developing cervical neoplasia and carcinoma.

• Preliminary studies suggested that HPV mRNA testing may represent a valuable biomarker in prevention of cervical cancer as an adjunct to cytology.
p16 histology

- p16 expression is related to HPV E7 activity
- Distinguish atypical squamous metaplasia from CIN3
- Adjudicate pathology results

Wentzensen 2007 Dis Markers
Bergeron 2010 Am J Surg Path
**p16 cytology for triage of HPV+ women (Italian screening trial)**

<table>
<thead>
<tr>
<th>Age 25-34 years</th>
<th>Relative sensitivity for CIN2+ (95% CI)</th>
<th>Relative sensitivity for CIN3+ (95% CI)</th>
<th>Relative referral to colposcopy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV testing (≥1 pg/mL) with no triage</td>
<td>3.50 (3.11-5.82)*</td>
<td>2.61 (1.21-5.61)*</td>
<td>3.64 (3.17-4.19)</td>
</tr>
<tr>
<td>HPV testing (≥1 pg/mL) and p16 triage (1+ cells stained)</td>
<td>3.01 (1.82-5.17)</td>
<td>2.52 (1.18-5.78)</td>
<td>1.15 (0.96-1.37)</td>
</tr>
<tr>
<td>HPV testing (≥1 pg/mL) and p16 triage (≥5% cells stained)</td>
<td>2.06 (1.20-3.68)</td>
<td>1.84 (0.81-4.38)</td>
<td>0.58 (0.46-0.73)</td>
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<th>Age 35-60 years</th>
<th>Relative sensitivity for CIN2+ (95% CI)</th>
<th>Relative sensitivity for CIN3+ (95% CI)</th>
<th>Relative referral to colposcopy (95% CI)</th>
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<tr>
<td>HPV testing (≥1 pg/mL) with no triage</td>
<td>1.63 (1.25-2.12)*</td>
<td>1.52 (1.06-2.19)*</td>
<td>2.38 (2.21-2.57)</td>
</tr>
<tr>
<td>HPV testing (≥1 pg/mL) and p16 triage (1+ cells stained)</td>
<td>1.53 (1.15-2.02)</td>
<td>1.32 (0.88-1.95)</td>
<td>1.08 (0.96-1.21)</td>
</tr>
<tr>
<td>HPV testing (≥1 pg/mL) and p16 triage (≥5% cells stained)</td>
<td>1.06 (0.73-1.49)</td>
<td>0.90 (0.52-1.45)</td>
<td>0.55 (0.46-0.64)</td>
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- Combined HPV+p16 testing has higher sensitivity than cytology, but much lower referral to colposcopy than HPV alone

Carozzi 2008 Lancet Oncology
The improved understanding of the molecular pathways of HPV-induced cervical carcinogenesis has led to the discovery of additional clinically useful biomarkers, many of which are proteins involved in the regulation of the cell cycle.

The detection of abnormally increased levels of these proteins may reflect the deregulation of the cell cycle in cervical neoplasia.

Other protein biomarkers, including p16 INK4a, Cyclin E, the MCM proteins, and TOP2A, have been used to detect the cells on a Pap test most likely to represent true cervical dysplasia or cancer.
• The recent **introduction of prophylactic HPV vaccines** will eventually **reduce the incidence of cervical cancers and its malignant precursors**, therefore **increasing the importance of biomarkers** in future cervical cancer screening programs to identify for treatment only those women truly at high risk for developing cervical cancer.

• The **use of these biomarkers and immunocytochemistry assays** can be applied both as a reflex test from an atypical Pap specimen but also as a primary screen to improve the **overall accuracy of the Pap test**.

International project of bilateral cooperation Romania-Hungary, National Programme II, Capacity Module III, 2008-2009, No. 54

EPIDEMIOLOGICAL MODELING OF THE HUMAN PAPILLOMA VIRUS INFECTION AS A RISK FACTOR IN THE CERVIS UTERI CARCINOMA CSONGRAD AND TIMIS COUNTIES

Workshop I
Sharing Experience and Action on Cervical Cancer Screening
18-19.10.2008, Timisoara, ROMANIA

Workshop II
Current Knowledge of Cervical Cancer and HPV
15-16.11.2008, Timisoara, ROMANIA
Management of Women with Atypical Squamous Cells of Undetermined Significance (ASC-US)

Repeat Cytology @ 6 & 12 mos
- Both Tests Negative
  → Routine Screening
- ≥ ASC (on either result)
  → Colposcopy
  Endocervical sampling preferred in women with no lesions, and those with unsatisfactory colposcopy
  - NO CIN
    - HPV Unknown
      → Repeat Cytology @ 12 mos
    - HPV Positive*
      → Cytology @ 6 & 12 mos or HPV DNA Testing @ 12 mos
  - CIN
    → Manage per ASCCP Guideline

HPV DNA Testing*
- Preferred if liquid-based cytology or co-collection available
- HPV Positive*
  - Managed in same manner as women with LSIL
  → Repeat Cytology @ 12 mos
- HPV Negative
  → Repeat Cytology @ 12 mos

Biomarker?
- HPV Unknown
- HPV Positive*

*Test only for high-risk (oncogenic) types of HPV
How to deal with multiple test results?

Management based on risk

- HSIL
- LSIL
- ASC-US
- HPV+/ASC-US
- HPV+/Cyto-
- HPV/-ASC-US
- HPV/Cyto-
- HPV16+
- HPV16-
- HPV16+ or HPV16+
- HPV16- & HPV18-
- Cyto-
- HPV16-
- Threshold for treatment?
Conclusions

1. Potential biomarkers for cervical cancer screening with a focus on the level of clinical evidence that supports their application as novel markers in refined cervical cancer screening programs.

2. Better markers indicating risk of precancer are neccessary.

3. HPV testing works and is being implemented in Romania – but not in the screening program, because it is expensive.

4. Vaccination will reduce the positive predictive value of all screening tests.

5. The management in the positive cases depends on the biomarkers.
Screening can save lives!

Biomarkers can improve it.