From cytology to full molecular cervical screening

Chris JLM. Meijer Dept of Pathology
Vrije Universiteit Medical Center
Amsterdam
The Netherlands
cjlm.meijer@vumc.nl
Cervical cancer worldwide

• Worldwide:
  – New cervical cancer cases 530,000/year
  – 3rd cancer in women
  – 275,000 women/year are dying of cervical cancer
  – 80% of cases in low resource countries: Africa, Mid- and South America and Eastern Europe

• Netherlands
  – Incidence: ASR/100,000  Mortality: ASR/100,000
    6.9                   1.6
  Absolute figures:
  • ~700 new cases/year  220 Death/year

Globocan IARC-WHO 2012
Current cervical screening tool in many countries: Pap test (cytology)

- Pap smear
- Liquid-based cytology (LBC)
Why should we change from cytology?
Problems in cervical screening by cytology

• Low sensitivity: many false pos. and false neg smears

• Frequent repeat testing necessary

• Subjective; moderate reproducibility

• Require good training of technicians and strong QC

• Not all women are reached for cervical screening
Problems cytology-based cervical cancer screening programmes:

1. Suboptimal sensitivity of the Pap test for cervical precancer

Cuzick et al. Int J Cancer. 2006
2. Not all women are reached for cervical screening

- In the Netherlands: 75% of women is protected (programmed & opportunistic)

- 25% is not screened at all (non-responders)
  - 57% of carcinomas in this group
Novel opportunity for cervical screening:

Testing for hrHPV presence

Q: Role of HPV in cervical carcinogenesis?
1. Persistent infection with hrHPV necessary for cervical carcinogenesis

2. No HPV, no cancer

3. 14 hrHPV types responsible for >99% of all CxCa: HPV 16 and 18 cause ~70% of all CxCa
Take home message

HPV testing vs cytology

HPV testing is more sensitive for CIN2+ detection than cytology; more objective

HPV provides better protection against CIN3 and cancer than cytology after a screen negative test

For screening purposes HPV testing is as good as HPV & cytology (Combo)

The HPV test is a more sensitive screening tool than the Pap test

HPV testing detects more CIN2+ than the Pap test

Arbyn et al., Vaccine 2012
Performance HPV & Pap (combo) vs HPV test alone

CIN2+

<table>
<thead>
<tr>
<th>Study</th>
<th>HPV alone</th>
<th>HPV&amp;cytology</th>
<th>DRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ronco, 2006</td>
<td></td>
<td></td>
<td>1.03 (0.74,1.42)</td>
</tr>
<tr>
<td>Mayrand, 2007</td>
<td></td>
<td></td>
<td>1.05 (0.57,1.93)</td>
</tr>
<tr>
<td>Kitchener, 2009</td>
<td></td>
<td></td>
<td>1.07 (0.94,1.22)</td>
</tr>
<tr>
<td>Naucler, 2007</td>
<td></td>
<td></td>
<td>1.06 (0.81,1.37)</td>
</tr>
<tr>
<td>Rijkaart, 2012</td>
<td></td>
<td></td>
<td>1.06 (0.89,1.26)</td>
</tr>
</tbody>
</table>

Overall (95% CI) 1.06 (0.97,1.16)

Sole HPV testing is nearly as sensitive as HPV&Pap: For screening use sole HPV testing

Arbyn et al., Vaccine 2012
Cumulative detection of invasive carcinoma
Pooled data from POBASCAM, NTCC, Artistic and Swedescree (600.000 women)

A negative HPV test provides better protection against cancer than cytology
Take home messages

- Women who were at enrolment HPV screen neg, have in the second round 50% less CIN3+ and significantly less cancer compared to women who were cytology screen negative at enrolment.

- HPV testing provides better protection against CIN3+ and CxCa than cytology.
Other advantage of HPV testing

- HPV testing can be done on self-collected cervico/vaginal material
Offering self-sampling for HPV testing to non-attendees

1. Can offering self-sampling for HPV testing increase compliance to screening?

2. Is this approach effective in detecting CIN2+?
## Offering self-sampling for HPV testing re-attracts non-attendees

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Method (self vs clinician)</th>
<th>Attendance rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gok et al. (2010)</td>
<td>Self-sampling vs recall letter (99:1)</td>
<td>Self-sampling (Delphi Screener) vs cervical smear</td>
<td>Self: 27.7%</td>
</tr>
<tr>
<td></td>
<td>28,073 non-responders</td>
<td></td>
<td>Recall letter: 16.6% P&lt;0.001</td>
</tr>
<tr>
<td>Gok et al. (2011)</td>
<td>Self-sampling vs recall letter (99:1)</td>
<td>Self-sampling (VibaBrush) vs cervical smear</td>
<td>Self: 30.8%</td>
</tr>
<tr>
<td></td>
<td>26,409 non-responders</td>
<td></td>
<td>Recall letter: 6.5% P&lt;0.001</td>
</tr>
<tr>
<td>Bais et al. (2007)</td>
<td>Self-sampling vs recall letter (9:1)</td>
<td>Self-sampling (VibaBrush) vs cervical smear</td>
<td>Self: 34.2%</td>
</tr>
<tr>
<td></td>
<td>2830 non-responders</td>
<td></td>
<td>Recall letter: 17.6% P&lt;0.001</td>
</tr>
<tr>
<td>Sanner et al. (2009)</td>
<td>Self-sampling (no control group)</td>
<td>Self-sampling (Qvintip) on demand</td>
<td>Self: 39.1%</td>
</tr>
<tr>
<td></td>
<td>2829 non-responders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virtanen et al. (2011)</td>
<td>Self-sampling vs recall letter (1:2.7)</td>
<td>Self-sampling (Delphi Screener) vs cervical smear</td>
<td>Self: 29.8%</td>
</tr>
<tr>
<td></td>
<td>4160 non-responders</td>
<td></td>
<td>Recall letter: 26.2% P = 0.02</td>
</tr>
<tr>
<td>Virtanen et al. (2011)</td>
<td>Self-sampling vs recall letter (1:2.7)</td>
<td>Self-sampling (Delphi Screener) vs cervical smear</td>
<td>Self: 31.5%</td>
</tr>
<tr>
<td></td>
<td>8699 non-responders</td>
<td></td>
<td>Recall letter: 25.9% P&lt;0.001</td>
</tr>
<tr>
<td>Szarewski et al. (2011)</td>
<td>Self-sampling vs recall letter (1:1)</td>
<td>Self-sampling (cotton swab, Qiagen) vs cervical smear</td>
<td>Self: 10.2%</td>
</tr>
<tr>
<td></td>
<td>3000 non-responders</td>
<td></td>
<td>Recall letter: 4.5% P&lt;0.001</td>
</tr>
<tr>
<td>Giorgi Rossi et al. (2011)</td>
<td>Self-sampling vs recall letter</td>
<td>Self-sampling (Delphi Screener) vs cervical smear</td>
<td>Self: 19.6%</td>
</tr>
<tr>
<td></td>
<td>2480 non-responders</td>
<td></td>
<td>Recall letter: 13.7% P=0.007</td>
</tr>
<tr>
<td>Wikström et al. (2011)</td>
<td>Self-sampling (n=2000) vs recall letter (n=2060)</td>
<td>Self-sampling (Qvintip) vs cervical smear</td>
<td>Self: 39.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Recall letter: 9.0% P&lt;0.001</td>
</tr>
</tbody>
</table>

Snijders et al Int J Cancer 2012
Two different self-sampling devices (used for hrHPV testing)

PROHTECT 1
N=~ 28,703 (age: 29-60 years)
Year of non-attendance: 2005
Delphi screener (cervico-vaginal lavage)

Gök et al., BMJ 2010

PROHTECT 2
N=~ 26,409 (age: 29-60 years)
Year of non-attendance: 2006
Viba brush (vaginal brush)

Gök et al., IntJCancer 2011
HPV self-sampling: a feasible and effective tool to screen non-attendees

Women invited to pap smear screening in 2005-2006 (n = 230 509)

Non-attendees (n = 54 482)
- Re-call
- Self-sampling (n = 52 447)
- Non-eligible (n = 1 497)

Screening participants (n = 176 027)

HPV-testing on self-samples (n = 15 274) (29%)

Histology after 18 months
- CIN2+ 1.4%
  - CIN2 n = 61 (0.4%)
  - CIN3 n = 144 (0.9%)
  - Cancer n = 13 (0.09%)

Histology after 18 months
- CIN2+ 0.8%
  - CIN2 n = 540 (0.3%)
  - CIN3 n = 941 (0.5%)
  - Cancer n = 59 (0.03%)
HPV testing in cervical screening

• HPV testing on self-collected c/v specimen is more sensitive than cytology in detecting CIN2+

• HPV testing on self-collected c/v specimen is as sensitive as HPV testing on physician taken smears, provided a clinically validated combination of a self-sampling device and a hrHPV test is used

Snijders Int J Cancer 2013; Arbyn Lancet Oncology 2014
HPV testing in cervical screening

- HPV vs cytology
- Clinical validation of HPV tests
- Triage of HPV pos women
HPV tests vary in their property to detect the various types of HPV infections

Important distinctions:

• Analytical sensitivity and specificity
  ➢ Detect all hrHPV infections: both transient (irrelevant) and transforming infections

• Clinical sensitivity and specificity
  ➢ Detect mainly HPV infections associated with CIN2+/3+ (clinically relevant hrHPV infections):
For HPV testing in cervical screening clinical validation is necessary

For screening purposes it is imperative to detect transforming HPV infections associated with (pre)cancer i.e CIN2, CIN3, CxCa and ignore the transient HPV infections

Otherwise too many women without lesions enter into diagnostic evaluation. Increase COSTS!

- Clinical validation of HPV tests obligatory!
- International guidelines have been formulated
Example: Case-control study: women with CIN3 vs women with normal cytology (≥30 years) and no CIN2+ in next 2 years

In women with normal cytology false positivity rate of a clinically non-validated test was significantly higher than that of a clinically validated test; true positive CIN3+ rate is similar.

Result: Unnecessary F-up, expensive, harmful, and overtreatment of women

Hesselink et al., 2008
Clinical validation of other HPV assays

• In order to become validated for use in cervical screening candidate HPV assays should prove:
  – their value in large prospective screening studies
  or
  – non-inferiority to validated reference assays (HC2 or GP5+/6+-PCR) in cross-sectional clinical equivalence studies

• Consensus guidelines for test requirements have been developed by an international consortium

  (Meijer et al. : Int J Cancer, 2009)
Clinically validated HPV assays for cervical screening

Available HPV detection assays

Many (>40)
- Hybrid Capture 2
- Diassay (GP5+/6+-PCR)
- COBAS4800
- APTIMA
- HPV RealTime
- SPF10
- Amplicor
- Cervista
- PapilloCheck
- PGMY
- … (and so on)

HPV tests validated for cervical screening (cervical scrapings)

- Hybrid Capture 2*
- Diassay (GP5+/6+-PCR)*
  - COBAS4800**
  - HPV RealTime**
  - PapilloCheck**
    - APTIMA**#
    - HPV-Risk assay**

HPV tests validated for cervical vaginal lavages (Delphi-screener)

- Diassay (GP5+/6+-PCR)
- HPV-Risk assay

*Based on longitudinal studies

**Based on equivalence analysis according to guidelines

# Provided that data of long term NPV of mRNA testing become available
HPV testing in cervical screening

- HPV vs cytology
- Clinical validation of HPV tests
- Triage of HPV pos women
HPV testing recognizes viral infection, but we need to detect disease.

HPV Testing (risk population)

Women

HPV DNA test

HPV + Women

Population at risk CxCa

Detection women at RISK

Even a clinically validated HPV test detects still both transient and clinically relevant infections.

We are only interested in HPV infections associated with disease: high grade lesions and cancer.
HPV testing recognizes viral infection, but we need to detect disease: triage testing necessary

HPV Testing (risk population)
Women
HPV DNA test
HPV +
Women
Population at risk CxCa
Detection women at RISK

20% precursor lesions
Low grade lesions
80%
No lesions

2-3 years

10-12 years

Carcinoma
High grade lesions
Clinically relevant HPV infections
1-3% cancer

TRIAGE (disease)
Detection of women with disease in need of Referral

Population with disease
Evaluation of triage tests in longitudinal studies (VUSA-Screen and POBASCAM)

- Cytology
- HPV 16/18 genotyping
- Combinations of these tests

➢ Aim to increase specificity without losing sensitivity

Rijkaart et al Int.J Cancer 2011; Dijkstra et al CEBP 2013
Katki et al Lancet oncology 2013
Presently two triage strategies have been adopted, because they are easy to implement and fulfill CIN3+ risk requirements (NPV > 98%)
A) Baseline cytology and cytology in follow-up (6 or 12 months)
B) Baseline cytology & HPV16/18 genotyping and cytology in follow-up (6 or 12 months)

Take home message

- The exact algorithm to be used for triage depends on the quality of cytology and the minimum positive predictive value for CIN3+ referral acceptable by local health decision makers (resources available)
alternative triage tests

- p16\textsuperscript{INK4A}/Ki67 dual staining
- Analysis Chromosomal alterations (eg 3q gain)
- Methylation analysis viral DNA
- Methylation analysis host cell genes
Methylation and Cancer

• Promoter methylation common event in cancer development to silence genes

• Promoter methylation of three tumor suppressor genes is functionally involved in cervical carcinogenesis
  - CADM1
  - MAL
  - miR-124-2

• Methylation levels of these genes increase with disease progression and are extremely high in CxCa


Bierkens et al. IJC 2013
Methylation assay detects cervical cancer and advanced CIN2/3 lesions

- Methylation levels increase with the severity of the lesion and duration of HPV infection
  
  \[\text{Bierkens et al. IJC 2013}\]

- Methylation levels are extremely high in cervical cancer: no cancers missed
  
  \[\text{De Strooper JCP 2014}\]

- Cin2/3 lesions detected by methylation are complementary to Lesions detected by cytology or HPV 16/18 genotyping
  
  \[\text{Verhoef Gyn.Oncology 2014}\]

Methylation markers: \textit{CADM1/Mal} and \textit{MAL/miR}
Methylation levels increase with the severity of the lesion and duration of HPV infection.

Methylation levels are extremely high in cervical cancer: no cancers missed.
Methylation marker of TSGs involved in cervical carcinogenesis

Concept supported by data:

**Cytology:**
Detects prevalent lesions (early and advanced) with reduced sensitivity for CIN3 and CxCa (sensitivity ~65% at best)

**Methylation marker panel:**
Detects advanced CIN lesions with high sensitivity: carcinoma proof (n=144)

- Methylation marker analysis (cut-off 70% specificity for CN3) and cytology are complementary in detection CIN3+ in hrHPV pos women: high sensitivity (~90%), low referral rate (~50%)

*Bierkens et al Int.J.Cancer 2013; Hesselink et al: submitted*
CADM1/MAL methylation analysis and cytology combined (CIN3+ outcome, physician taken scrapes)
PreCursor-M kit (CE/IVD certified): quantitative multiplex methylation-specific PCR for CADM1, MAL, and miR-124-2

Snellenberg et al., 2012

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N=</th>
<th>Methylation positivity in cervical scrapes</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;=CIN1</td>
<td>209</td>
<td>26%</td>
</tr>
<tr>
<td>CIN2</td>
<td>32</td>
<td>31%</td>
</tr>
<tr>
<td>CIN3</td>
<td>60</td>
<td>75%</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>67</td>
<td>100%</td>
</tr>
</tbody>
</table>

In cervical scrapes the PreCursor-M kit detects all carcinomas.
Summary on physician taken smears

• CADM1 and MAL (miR124-2) methylation analysis is an alternative or complementary triage tool to cytology for HPV positive women

• Sensitivity particularly high for advanced CIN2/3 and cervical cancer in need of treatment
Performance PreCursor-M kit in lavage self-samples

Also in lavage self-samples the PreCursor-M kit detects all carcinomas
Direct triage of HPV-positive women by PreCursor-M test makes objective and full molecular cervical screening possible.
Present International situation cervical screening by HPV

Primary HPV Screening will be implemented in

**The Netherlands**: Jan 2016
- Women 30-60 years, 30,35,40, 50,60y. Triage with cytology at baseline and 6 months.
- If HPV screen pos and triage test neg at 40,50, or 60y: repeat testing after 5 years
- Non-responder women are offered opt-in for HPV self-sampling

**Australia**: advice medical services advisory committee 4/04/2014:
- Start primary HPV screening
- Women: 25-69 years, 5 years interval, Triage by cytology and HPV 16/18 genotyping at baseline and cytology at 12 month

**Italy**: 5 regions start HPV screening in 2015
- Women 25-65 y, 5 years interval, Triage by cytology and HPV 16/18 genotyping

**Nordic countries**: are considering or doing implementation pilot studies

www.gr.nl;

www.msac.gov.au
Acknowledgements

VU University Medical Center (VUmc)

Department of Pathology
- P. Snijders
- D. Heideman
- F. van Kemenade
- L. Rozendaal
- M. Gök
- B. Hesselink
- R. Steenbergen
- S. Wilting
- V. Verhoef
- M. Uijterwaal
- M. dijkstra

Department of clinical epidemiology and biostatistics
- N. Fransen
- M. Verkuyten
- D. Boon
- M. Lettink
- F. Topal
- D. Buma
- M. Bogaarts
- R. van Andel
- R. Pol
- M. Doeleman

Gynaecologic Oncology
- G. Kenter

EEC consortia
- PreHDICT
- CoHeaHr
- Mass-care

Dutch Cancer foundation
ZON-MW
Thank You

Merci

Salamat

Kop

Hvila

Diolch

Kiitos

Sheun

Shnorhakalu

Gamsahapnida

Dank

Takk

Krap

Tack

Grazzi raibh

Blagodariya

Gomapsupnida

Kun

Danke

Enkosi

Terima

Dhanyavad

Dhanyavaadaluu

Dakujen

Daw Waad

Khopjai

Kruthagnathalu

Danke

Kasih

Mamnoon

Ngiyabonga

Cam

Dzie

Shokrun

Spaas Mul

Al or

Dankie

Gracie

Grazi

Dhonnobaad

Asante

Hain

Dhan

daa