HPV and cervical screening

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Burden of disease

- Cervical screening reduces incidence/death rate from carcinoma of cervix.
- Incorporation of organised call/recall in quality assured environment appears ideal to achieve these outcomes.
Cervical cancer incidence rates per 100,000 women, England and Wales, 1971-98

Rate per 100,000 women (age-specific)

Year of death

Rate per 100,000 women (all ages)*

* European age standardised rate

Year of death


25 - 34
35 - 44
45 - 54
55 - 64
65 - 74
75+
all ages*
Welsh cancer data

- Average registrations per annum: 174
- Rank: 13th
- Average deaths per annum: 70

*Cancer in Wales, 1995-2009: A comprehensive report*
Priorities for successful cervical cancer screening improved coverage/improved sensitivity expected with HPV-based screening.

Five yearly coverage

<table>
<thead>
<tr>
<th>Age</th>
<th>2005/6</th>
<th>2006/7</th>
<th>2007/8</th>
<th>2008/9</th>
<th>2009/10</th>
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<tbody>
<tr>
<td>25-49</td>
<td>79.5%</td>
<td>79.2%</td>
<td>78.6%</td>
<td>78.9%</td>
<td>78.9%</td>
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<td>50-64</td>
<td>80.7%</td>
<td>80.5%</td>
<td>80.3%</td>
<td>80.0%</td>
<td>78.9%</td>
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<tr>
<td>All-Wales 25-64</td>
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<td>79.7%</td>
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NHS Cervical Screening Programme Annual Review 2010
KC53 Statistical Report, Cervical Screening Wales 2010
Papillomavirus – phylogenetics

HPV infection is very common

Where does HPV testing fit into the screening programme?

...some HPV background

HPV testing

• **HR-HPV highly prevalent (up to 33%) in sexually active women <30yr.**
• **80% clear in 12-18 months.**
• **HC II test oncogenic types (>5000 copies/ cell)**
  – 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.
• **PCR consensus primer tests for same types but extremely high sensitivity (<10 copies/ cell)**
  – less useful due to X-specimen contamination.
HC II higher sensitivity/ lower specificity than conventional cytology (Clavel et al, 1999; Schiffman et al, 2000; Mansell et al, 1994)

<table>
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<th></th>
<th>sensitivity (%)</th>
<th>specificity (%)</th>
<th>PPV (%)</th>
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| *moderate dyskaryosis or worse predicting underlying CIN II+* (Dobbs and Ireland, 2000)

- Cytology (high grade): 77.4 - 85.3
- HPV (high risk): 100 - 88.4
  - Specificity: 94.9
  - PPV: 74*
  - Specificity: 85.2 - 55
  - PPV: 55
**HPV vaccination**

- Vaccines protect against approximately 50% of pre-invasive cervical disease (*Smith et al, 2007*).
- May be cost effective in screening setting if screening intervals lengthened (*Goldie, 2003*)
  - but duration of vaccine protection/ need for boosters unknown.
- Vaccination should have protective effect for cervical adenocarcinoma not seen for cytology
  - may not be seen for HPV based screening (*Collins et al, 2006*).
- HPV associated with majority of vaginal/ anal carcinomas, around 50% of vulval carcinoma and about 30% of oesophageal carcinoma (*Collins et al, 2006*)
  - value of HPV immunisation for non-cervical HPV related carcinomas unclear.
Oncogenic HPV types in cervical cancer – all world regions

Effect of vaccination upon colposcopic practice difficult to determine.

Incidence HG CIN will be reduced.

PPV will decline as incidence of disease drops (Franco et al, 2006).

Depending upon type of vaccine used amount LG disease should be reduced.

With continued cytology based screening about 25% of women with LG cytology harbour HG CIN. 1/2 protected by vaccination and would not be referred to colposcopy

- ie if vaccination is used with bivalent vaccine and cytology based screening programme approx.12.5% of current LG cases would not prevented by vaccination and have CIN2+.

If quadrivalent vaccine used the number of HG cases referred to colposcopy unchanged but referral rate to colposcopy reduced due to protection against viral types 6/11 and by implication LG CIN.
HPV test of cure (ToC)

- Meta-analysis of 5 studies including 1032 women treated by excision CIN2/3
  - sensitivity for HCII to detect CIN2+ 90.7% vs. 76.6% for cytology (threshold ASCUS)
  - specificity of 74.6% vs. 89.7% (Chan et al, 2009).
- Time of testing between 3-6 months.
- Incidence recurrent CIN2+ 6.6%.
- HPV testing represented 2x increase in rate of referral back to colposcopy compared to cytology based follow-up
  - approx. 15% increase in detection recurrent high grade CIN.
- If combined HPV testing/ cytology for follow up then sensitivity remained @ 90.7% but specificity increased to 93.1%.
- Benefit is also a rapid return to normal recall for HPV –ve cases.
Suggested Algorithm for Test of Cure
(modified from NHSCSP protocol Aug 2011)

Treatment

6 month follow up

Cytology

Moderate dyskaryosis or worse

Colposcopy
Approx 18-25% of all treatments

Treat, or if N, for cytology follow up according to national guidelines

Normal, borderline or mild dyskaryosis

Cytology

HPV +ve

Normal recall
2.9% CIN2+ @ 3 years*

HPV –ve

Questions

• No evidence to guide management following treatment for cGIN
• If colp N with negative/ low grade cytology and HPV +ve (regardless whether scj seen or not) then suggest repeat cyto/ HPV testing @ 6 months but no clear guidance here.

*Kitchener et al, 2008
HPV triage

- For women with ASCUS/LSIL cytology risk of CIN2+ appears equivalent in HPV +ve women whether accompanying cytology ASCUS or LSIL from ALTS data @ 23% (ALTS Group, 2003a and b; Guido et al, 2003).

- Meta-analysis 16 studies HCII appears more effective than cytology with ASCUS to detect CIN2+ with 14% improved sensitivity over repeat cytology for similar specificity (Arbyn et al, 2005).

- LSIL does not benefit from HPV triage due to high incidence of HPV positivity in this group
  - has been disputed (Kelly et al, 2011a).
  - identification of infection with HPV types 16/18 or multiple infections involving HPV 16 (Spinillo et al, 2009) may further refine HPV based triage of minor cytological abnormalities.
• ATHENA study of 47,208 looking at utility of HPV-based screening noted HPV16/18 testing for ASCUS cytology improves detection of CIN2+ in 1,923 women >21 years of age
  – 24.4% vs. 14.0% for HR HPV and vs. 0.8% for HPV –ve.
• Using modified and clinically useful form of PCR.
• RR CIN2+ for HPV16+ve vs. non-HPV16/18 was 3.7 and RR for CIN3+ was 4.5 (Stoler et al, 2011b).
• 2 yr cumulative risk CIN2+ from ALTS for HPV16 with ASCUS/ LSIL cytology was 50.6%
  – risk for women infected with HR-HPV non-16 was 4.7 - 29.5% depending upon HPV type (Wheeler et al 2006).
• All HR-HPV in this setting requires colposcopy but those with HPV 16 and normal satisfactory colposcopy should not be considered for return to normal recall.
• Cumulative incidence CIN2+ of 4.4% for HPV +ve women @ 3 yrs after normal/ satisfactory colposcopy low enough with preceding borderline/ mild dyskaryosis to be returned to normal recall (Kelly et al, 2011).
Suggested Algorithm for Triage of Low Grade Abnormalities
For cytology based screening

- Cytology b/line, b/line HG or b/line endocervical
  - Negative
    - Normal recall
  - Mild dyskaryosis+
    - Colposcopy
  - HPV test
  - HPV -ve
    - Normal recall
  - HPV +ve
    - Consider HPV 16 typing
      - HPV 16-ve
        - Normal recall
      - HPV 16+ve
        - HPV 16 abnormal, biopsy CIN1
          - Colposcopy normal and satisfactory
          - Repeat HPV testing and cytology @ 6 or 12 months
        - HPV 16+ ve
          - Colposcopy abnormal, biopsy CIN2+
            - Offer treatment

Questions
- If colp unsatisfactory but otherwise N after LG referral then suggest repeat cyto/ HPV testing @ 6 or 12 months.
- If HPV 16+ but colposcopy N suggest repeat HPV testing/ cytology @ 6 or 12 months.
**HPV-based screening**

- Pooled data from 11 case controlled studies revealed OR HPV16 preceding cervical squamous carcinoma was 434.5 using GP 5+/6+ primer from 1739 cases
  - HPV DNA extracted from 96.6% of cases /15.6% of controls (Munoz et al, 2003).
  - HPV16 commonest HR-HPV type in cancer cases in all 4 continents from which cases obtained.
- Primary HPV screening for cervical cancer superior to cytology-based screening
  - in detecting CIN3+ lesions (Arbyn et al, 2009)
  - in reducing incidence cervical cancer (Ronco et al, 2010).
• *Most randomized controlled trials/pilot projects used either co-testing or HPV testing alone with cytology triage*
  referred for colposcopy if:
  – HR-HPV +ve women with abnormal cervical cytology
  – women who show HPV persistency.

• *Koliopoulos et al (2006) - meta-analysis of 25 studies reported combined sensitivity of HCII HPV testing to detect CIN2+ of 90.0% compared to cytology (threshold ASCUS) of 72.7%*
  – sensitivity for HPV testing increased to 94.8% for women >30 years
  – all but 1 study cross sectional.

• *Specificity for CIN2+ poorer for HPV testing against cytology (86.5% vs 91.9%; threshold of ASCUS).*
• **Arbyn et al (2006)** found sensitivity for HCII of 97.9% in further meta-analysis with pooled specificity of 91.3%.

• *Combined HPV/ cytology testing in screening setting would not improve detection rate of CIN3*+ (Dillner et al, 2008) *and would have an unacceptable false +ve rate*
  
  – test +ve rate for cytology varied considerably (Dillner et al, 2008) in different countries not explained by prevalence HPV
  
  – poor sensitivity in some countries for cytology inflating benefit of HPV-based screening.

  – NPV HPV-based screening approaches 100%.

• **5 yr disease free rate approaches 2 year disease free rate for –ve cytology** *(Kjaer 2004)*
  
  – alternatively 40-50% better than cytology (Naucher et al, 2007; Bulkmans et al, 2007; Sherman et al, 2003)
  
  – providing basis to lengthening screening interval with HPV-based screening.

• **Women <30 yrs of age specificity HPV-testing unacceptable due to high prevalence of HPV infections** *(Kitchener et al, 2004; Peto et al, 2004; Cuzick et al, 2006; Dillner et al, 2008)*
  
  – of which many are transient/ of no clinical concern.
• More colposcopy referrals would be anticipated with HPV-based screening.
• If screening becomes HPV based then women with low risk HPV infection should not be referred for colposcopy.
• Cumulative risk @ 14 yrs for CIN3 among cytologically normal women with HR-HPV at entry was 28% with half of these women detected at next screening round in longitudinal study of 7,278 women (Peto et al, 2004).
• Prevalence of CIN3 highest in this study in those with HPV16 with OR 3-4x higher than for other HPV types.
• HPV16 also >2x as likely to persist than any other HPV type.
• Long term follow up shows cumulative risk of CIN3+ with cyto–ve HPV16+ve @ 10 yrs of 20.7%
  – 17.7% for HPV18+ and 1.5% for HR-HPV -ve (Khan et al, 2005).
• Other HR-HPV types have absolute risk CIN3 approx. 6% @ 12 yrs follow up (Kjaer et al, 2010).
• ATHENA study reported on 4,219 who were HR-HPV +ve but cytology -ve in women of ≥30 yrs of age.
• Prevalent CIN2+ rate on those who were HPV 16/18+ve was 11.4%
  – rate for those HR HPV+ve (not HPV16/18 +ve) was 6.1%
  – and those who were HPV -ve was 0.8% (Wright et al, 2011).
• ARTISTIC data revealed HPV16/18 present in 2.2% of normal cytology (Kitchener et al, 2006).
• Castle et al (2007) considered colposcopy appropriate if cumulative risk of CIN3+ over 2 yrs at least 10%.
• ATHENA study **prevalent rate** CIN3+ for -ve cytology and HPV16/18+ve of 9.8% and therefore colposcopy appears appropriate in this setting.
• If HR-HPV+ve (HPV16/18 -ve) then repeat cytology/ HPV testing in 12 months.
• If so cytology may become redundant to triage HPV +ve cases in screening setting.
Suggested Algorithm for HPV-Based Screening

Screen with HPV testing from 30 years. Consider offering 2 rounds of cytology from 25 years

Questions
- Test +ve rate 9-13% (Arbyn et al, 2009)
- Upper age of screening 60 or 65 years?
• HPV testing reproducible, objective/ easily automated.
• Inherent low sensitivity of cytology means that many tests from well screened women may lead to delayed diagnosis of CIN/ cancer.
• HPV testing valid screening method appears at least as effective as cytology (IARC, 2005) which can also detect cGIN/ adenocarcinoma (Castle et al, 2011).
• HPV testing likely to increase numbers of cases requiring colposcopic assessment but detect more CIN3 (Peto et al, 2004).
Biomarkers

• 80% population exposed to HPV with 10% persistent /risk of transforming host genome
  – marker for DNA incorporation would be useful.
• Instead of testing HPV DNA as HC II, HPV mRNA for E6/7 may be predictive of infections to lead to high grade dysplasia/ cancer.
• HPV Proofer kit being developed for types 16, 18, 31, 33, 45 mRNA
  – no performance data available.
• Differential expression host cell cycle regulatory proteins may have role as biomarkers for dysplasia.
• 3 markers shown promise
  - CDC6 DNA replication licensing protein
  - MCM5 DNA replication licensing protein
  - p16\textsuperscript{INK4} cyclin-dependant kinase inhibitor.
• **MCM5** - minichromosome maintenance protein licenses chromosomal replication at specific chromosomal sites.

• **CDC6** - cell division cycle protein loading factor assembling MCM’s onto chromosomes prior to mitosis.

• **CDC6** (E6/p53 related) and **MCM5** (E7/pRb related) are overexpressed in dysplasia/ carcinoma.
  – allows continued cell replication.

• **p16^INK4^** - tumour suppressor protein overexpressed due to loss of −ve feedback by E7 mediated inactivation of pRb
  – can identify dysplasia/ carcinoma.

• **p16^INK4^** also +ve in TEM/ endosalpingiosis.

• All detected in LBC slides or h/p.
The diagram illustrates the molecular mechanisms involved in HPV-induced tumour suppression, focusing on the G1 phase of the cell cycle.

1. **MCM5/p16**: This protein is involved in the regulation of the cell cycle, specifically at the G1/S transition. A decrease in MCM5/p16 expression has been observed in HPV-induced tumours.
2. **Cyclin D + CDK4,6**: These proteins are involved in the Rb/E2F pathway, which is activated by HPV E7 oncoprotein. The increased expression of Cyclin D leads to the release of E2F from Rb, allowing E2F to promote cell cycle progression.
3. **Cyclin E increased**: This results in the activation of E2F, which promotes DNA synthesis (S-phase).
4. **P-Rb**: Phosphorylation of Rb by Cyclin D/CDK4,6 results in the release of E2F, leading to the expression of genes involved in DNA synthesis.
5. **Abrogates G1/S checkpoint**: This step indicates that the G1/S transition is compromised due to the HPV E7 oncoprotein, allowing cells to progress through S-phase with decreased regulation.
6. **Aberrant S-phase induction**: The lack of regulation at the G1/S checkpoint leads to aberrant DNA synthesis.
7. **Telomerase expression**: The HPV E6 oncoprotein enhances telomerase expression, which is essential for maintaining telomere length and cell viability.
8. **hTERT transcription**: HPV E6 also upregulates hTERT transcription, further stabilizing telomeres.
9. **Mdm2**: This protein binds p53, leading to its degradation. This is facilitated by the HPV E6 oncoprotein, which downregulates Mdm2 expression.
10. **Cyclin A, B + CDK1, CDK2**: These proteins are involved in the G2/M transition and are regulated by HPV E6, leading to the expression of S-phase genes and DNA replication.

The diagram also highlights the role of SMAD 2, 3, and 4 in the TGF-β tumour suppressor pathway, which is disrupted by HPV E7, further contributing to the uncontrolled cell cycle progression seen in HPV-induced tumours.
Other biomarkers

- *Brn-3a mRNA indicates activation of E6/7 as transcription factor.*
- *Ki-67, PCNA, HTERT unreliable.*
- **Advantage of biomarker panels when added to cytology is results could alter cytologic diagnosis**
  - eg borderline grades re-classified normal, low or high grade
  - HPV testing unable to alter cytologic result.
- **Biomarkers may have role with cytology in screening/ triage.**
Conclusion

HG cytology

LG cytology

HPV +ve

HPV -ve

For b/line.
For mild too?

Recurrent inad cytology

If treatment

Cytology or HPV +ve

Cytology -ve/ HPV -ve

Normal recall

Low risk of CIN 2+
(Kitchener et al, 2008)
Post vaccinated era

- HPV test +ve for >30 yrs
  - +/- Biomarkers
    - 16/18 or cytology +ve
    - 16/18 or cytology -ve
      - HPV test @ 6 or 12 months
      - Normal recall
      - Cytology -ve/HPV -ve
      - Cytology or HPV +ve

If treatment