Defining the Impact of HPV on Cervical Cancer

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Epidemiology of HPV

- Virtually all humans are simultaneously colonized by several HPVs
- Many only cause asymptomatic infections
- Certain HPV strains lead to a wide range of clinical conditions:
  - Low-risk strains
    - Self-limited benign growths, e.g., warts, condyloma
  - High-risk strains
    - Cancers of cervix, vagina, vulva, penis, anus, oropharynx
HPV Prevalence

HPV Prevalence in North America by age, among women with negative cytology

Bruni et al. 2010
ETE module 1, ASCCP, 2016.
Cervical Cancer is Caused by HPV

- HPV acquired through sexual and genital skin-to-skin contact
- Prevalence peaks within few years of median sexual debut age (17 yo)
- >90% HPV infections transient and become undetectable in 1-2 y
- **Persistent HPV** predicts future development of CIN3+

Wright et al., 2003; ETE module 1, ASCCP, 2012
Human Papillomavirus

- 90% of CIN lesions and cervical cancer are associated with HPV
- Most frequent type in CIN 2, 3 and invasive squamous cell carcinoma is HPV 16, followed by HPV 18
- Classification
  - High-risk types: 16, 18, 31, 33, 51, 52, etc.
  - Low-risk types
  - Intermediate / indeterminant risk types (frequently this category is included in the high-risk types)
Human Papillomavirus

- Double-stranded circular DNA virus

- E6 Promotes immortalization by degrading p53
  Modifies cell adhesion and differentiation

- E7 Promotes pRb degradation permitting cell progression to S-phase
  Induces chromosomal instability

- L1 Major capsid protein
  Can auto-assemble into viral-like particles

- Early (E) region proteins enhance cellular replication and facilitate viral replication.
- Late (L) region encodes the capsid proteins.
HPV changes

• **Top:**
  - Typical koilocytic cells in HPV infection of uterine cervix
  - Note cavitation, binucleation and pyknotic nuclei

• **Bottom:**
  - Condyloma of the uterine cervix
  - Note koilocytes in superficial layers of epithelium and many binucleated cells
Annual Burden of HPV-associated Disease – U.S. Women

- 4,210 cervical cancer deaths
- 12,820 new cases of cervical cancer
- 330,000 new cases of HSIL: CIN2/3 (high grade cervical dysplasia)
- 1 million new cases of genital warts
- 1.4 million new cases of LSIL: CIN1 (low grade cervical dysplasia)

ACS 2016; Sex Transm Dis 2004; Schiffman et al., 2003
Declining Incidence of Cervical Cancer – Persistence of disparities

*National Cancer Institute’s Statistics, Epidemiology, and End Results (SEER) database

ETE module 1, ASCCP, 2012.

Prevalence: 2,274,000 women have cervical cancer

Incidence: 510,000 new cases each year

2000 estimated incidence of invasive cervical cancer by selected region

Mortality: Second leading cause of female cancer-related deaths (288,000 annually)

Decrease in Cervical Cancer Mortality Following Introduction of Pap Test in the United States

1. OBGYN.net. Available at: http://www.obgyn.net/displayarticle.asp?page=yw/articles/braun_PAP
Objectives of Screening

- Prevent morbidity and mortality from cervical cancer
- Prevent overzealous management of precursor lesions that most likely will regress or disappear and for which the risks of management outweigh the benefits
Abnormal Pap test – How common is it?

- 50-60 million women screened
- 2-3 million ASC (Atypical Squamous Lesions)
- 1.25 million LSIL (Low-Grade precancerous lesions)
- 300,000 HSIL (High-Grade precancerous lesions)
- 12,000 cancers
Natural History of Cervical Cancer

- HPV infection
- CIN 1
  - Avg. 6-24 mo.
- HPV disappearance
- CIN 2,3
  - Avg. 6-12 mo.
  - Avg. 10-13 yrs
- Invasive CA
Cervical cancer screening should begin at age 21.

Women < 21 should not be screened regardless of age of sexual onset

Guidelines do not apply to special populations – hx of cervical cancer, DES exposure, & immune-compromise

Saslow, Solomon, Lawson, et al. JLGTD, March 14, 2012 (online)
Screening for ages 21-29

- Cytology alone every 3 years
- HPV testing “should not be used to screen”
  - Not as a component of cotesting
  - Not as a primary stand-alone screen
Rationale for Avoiding HPV Tests Among Women Ages 21-29

- Prevalence of carcinogenic HPV approaches 20% in teens and early 20s

- Most carcinogenic HPV infections resolve without intervention

- Identifying carcinogenic HPV that will resolve leads to repeated call-back, anxiety, and interventions without benefit
Screening For Women Ages 30-64

- Cytology + HPV testing (Co-testing) every 5 years is preferred
- Cytology alone every 3 years is acceptable
Rationale for Co-testing, Ages 30-64

- Increased detection of prevalent CIN3
- Achieves risk of CIN3 equal to cytology alone @ 1-3 year intervals
- Enhances detection of adenocarcinoma/AIS
- Minimizes the increased number of colposcopies, thus it reduces harms.
When to Stop Screening

- Stop at age 65 for women with adequate negative prior screening, no CIN2+ within the last 20y.

- Definition of adequate negative screening:
  - 3 consecutive negative Paps or
  - 2 consecutive negative HPV tests
    - (Tests within 10 years of stopping; most recent within 5 years.)
Rationale for stopping at 65 years

- CIN2+ is rare after age 65
  - Most abnormal screens, even HPV+, are false + and do not reflect precancer
- HPV risk remains 5-10%
- Colposcopy/biopsy/treatment more difficult
  - Harms are magnified
- Incident HPV infection unlikely to lead to cancer within remaining lifetime

Chen HC et al. JNCI 2011;103:1387-96;
Rodrigues AC et al. JNCI 2009;101:721-8
When to stop screening

- Stop after hysterectomy with removal of cervix and no history of CIN2+

- “Evidence of adequate negative prior screening is not required”
When NOT to stop at age 65 years

- If history of CIN2, CIN3, or AIS
  - Continue “routine screening” for at least 20 years, “even if this extends screening past age 65.”
Use of HPV testing

- Use for ASCUS patients 21 and over for triage (i.e. “reflex” testing)
- No use for screening (i.e. co-testing with Pap and HPV) before age 30
- For women age 30 and over Pap/HPV co-testing may be used every 5 years as an alternative to pap alone every 3 years, if results are normal.
- Current US guidelines do not support primary screen with HPV alone, or for any reason in women younger than 21.
Gardasil® Vaccine

- Quadrivalent Human Papillomavirus recombinant vaccine
  - Serotypes 6, 11, 16, 18
  - HPV 16 and 18 → 70% of cervical cancer
  - HPV 6 and 11 → 90% of genital warts
- Ages 9 to 26
- Administered as 3 IM injections over 6 months

- GARDASIL 9
- helps protect against 9 types of HPV
- Types 6, 11, 16, 18, 31, 33, 45, 52, and 58
Major categories of cervical carcinoma

- Squamous cell carcinoma (most frequent)
  - Most are of the large cell, non-keratinizing type
- Adenocarcinomas
- Other
- Adenosquamous carcinoma
- Glassy cell carcinoma, etc.
Presentation

- Abnormal bleeding or brownish discharge, frequently following intercourse
- Often patients have a history of not having had a cytologic (Pap) smear for many years
- Other symptoms, such as back pain, loss of appetite, weight loss, pelvic pain/pressure, unilateral leg swelling or pain and a pelvic mass are late manifestations and occur when there is extensive spread of carcinoma
Plate 12-4. Papillary tumor of the cervix.
Plate 12-3. Mass on the anterior lip of cervix with atypical vessels and yellow appearance. There is also leukoplakia of the posterior lip of the cervix.
Plate 12-2. Fungating cancer with obliteration of the os and multiple atypical vessels.
Plate 12-1. Large, raised cancer, yellow in appearance, on the posterior lip of the cervix. There is an ulcer at 6 o’clock and atypical vessels throughout the mass.
Risk factors for cervical cancer and its precursor lesions

Demographic factors

- Age
- Dysplasia age 32 (low grade) – 38 (high grade)
- Carcinoma in situ age 42
- Invasive cancer age 52
- Race: Black/Hispanic/Native American
- Low socioeconomic status / educational level
Risk factors

- Behavioral and sexual factors
  - Large number of sexual partners
  - Early age at first intercourse
- Cigarette smoking
- Long-term contraceptive use
- Diet low in folate, carotene, vitamin C
Risk factors

Medical / gynecologic factors

- Multiparity
- Early age at first pregnancy
- History of STDs (especially HSV or HPV-associated lesions)
- Infection with specific types of HPV
- Lack of routine cytologic screening
- Immunosuppression (any cause)
What happens when patient has an abnormal Pap test?

- Depending on Pap test result, the provider may advise one or more of the following:
  - HPV testing
  - Repeat Pap
  - Colposcopy
  - Possibly an endometrial biopsy
  - Possible referral to gynecologic oncologist
System Failures Leading to Cervical Cancer Diagnosis

Patient does not get appropriate therapy

Health care providers do not screen women at visits

Women do not come in for screening

Patient gets cervical cancer

Colposcopy for abnormal screen not done

Courtesy of Connie Trimble, MD, Johns Hopkins University School of Medicine, Baltimore, MD
Diagnosis

- Abnormal cytologic screening (Papanicolaou smear) does not establish the diagnosis.
- If the cytologic exam is abnormal and no lesion is visible then a colposcopy is performed with biopsies and endocervical curettage.
- If no lesions can be identified on colposcopy and ECC is negative then a cervical conization should be performed (CKC, LEEP, LEETZ).
Colposcopy
Acetowhite lesions

Low grade CIN with faint acetowhite changes and geographic margins. The changes extend into the canal and the distal margin of the changes is not visualized.
Colposcopy: Acetowhite lesions and Punctuation
Colposcopy

Low grade CIN with faint acetowhite changes and geographic margins at 1 o’clock. An ectropion is present and metaplasia is seen at 10 and 12 o’clock.
Low grade CIN with faint acetowhite changes and geographic margins. The SCJ is visualized. A gland opening is noted at 11 o’clock.
Low grade CIN with shiny acetowhite changes and geographic margins. The SCJ is visualized.
Management of Abnormal Screening & Cervical Dysplasia

- Leads to more frequent surveillance
- CIN 2 or 3 treatment involves destructive or excisional procedures on cervix
- Current CIN 2 or 3 treatments are effective but can have negative consequences:
  - Anxiety
  - Pain
  - Bleeding
  - Costs
  - Future obstetric complications
  - Cervical stenosis or scarring
Risks of Current Treatments for High Grade Dysplasia

- **Reproductive Outcomes***
  - **Cervical stenosis:** 8% (0-27%) *Monteiro et al., 2008, Suh-Burgmann et al., 2000*
  - **Second trimester pregnancy loss:** RR 2.6 *Kyrgiou et al., 2014*
  - **PPROM:** RR 2.7 *Kyrgiou et al., 2006*
  - **Preterm delivery:** RR 1.7-2.15 *Kyrgiou et al., 2006, Weinmann et al., 2017*

  *Associated with depth of excision, mode of excision, pregnancy interval, postmenopausal status, and variability in comparison groups amongst studies (healthy controls vs untreated CIN)*

- **Psychosocial Outcomes**
  - **Anxiety**
  - **Pain**
  - **Bleeding**
  - **Need for additional interventions**
Therapeutic vaccination: An alternate strategy

- Immune-based therapy to clear CIN 2,3 without destruction of cervical tissue: therapeutic vaccination
- Current FDA-approved HPV vaccine is prophylactic – no therapeutic effect in patients with pre-existing HPV-associated cervical lesions

<table>
<thead>
<tr>
<th>HPV type</th>
<th>% Clearance (#cleared/total infections)</th>
<th>% Change in HPV Infection</th>
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<tbody>
<tr>
<td></td>
<td>HPV Vaccine</td>
<td>Control</td>
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<tr>
<td>HPV 16</td>
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<tr>
<td>6 mo</td>
<td>27.3</td>
<td>27.5</td>
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<td>12 mo</td>
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<td>45.9</td>
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<td>HPV 18</td>
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<tr>
<td>6 mo</td>
<td>46.1</td>
<td>44.7</td>
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<tr>
<td>12 mo</td>
<td>59.3</td>
<td>60.7</td>
</tr>
</tbody>
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Group FIS, 2007; Garland et al., 2007; Hildesheim et al., 2007
Why doesn’t the prophylactic HPV vaccine work against preexisting cancer?

- HPV vaccines induce antibodies to block HPV from entering the body
- Preexisting cancer cells cannot be eliminated by antibodies
- Cancer cells are killed by T cells
- HPV vaccines induce T cells, but T cells cannot find their way to get into the cervix
Challenges to developing a therapeutic vaccine

- Need to mount a systemic cellular immune response to HPV antigens.
- Requirement of migration of circulating effector T cells to the infected tissue.
  - Female reproductive tract is immunologically restrictive
  - Infection or inflammation needed to permit entry of systemic T cells
  - Establishment of tissue-resident memory cells to the cervix would be desirable for surveillance and killing of transformed cells.
“Prime and Pull” Strategy

• “Prime and Pull”: Highly effective vaccination strategy to establish tissue-resident memory T cells in the genital tract
  - Prime = initial systemic T cell response triggered by parenteral vaccination
  - Pull = activated T cells recruited into genital mucosa through application of specific chemokines; establish long term tissue-resident memory T cell pool
  - Reduces spread of infectious viruses and prevents clinical disease in mice

Shin, Iwasaki, Nature 2012
Our “Prime and Pull” Strategy

- Our proposed “prime and pull” strategy
  - Prime = initial systemic T cell response triggered by FDA-approved prophylactic 9-valent HPV vaccine
  - Pull = HPV-activated T cells recruited into cervical lesions with imiquimod, a topical immune response modulator
9-valent HPV Vaccine

- Protects against 7 high-risk HPV strains that cause 90% of cervical cancers
- Sub-unit protein (L1) vaccine with VLPs mimicking wild type virus capsid (contain no viral DNA)
- Highly immunogenic producing a robust antibody response
- Induces L1-specific T cell response as well
Pull; Imiquimod

- Agonist for TLR7
- Has antiviral and antitumor activity
- Induces cytokines: interferon, TNF, IL-1, IL-6, IL-9
- Activates T cells to trigger an immune response associated with HPV clearance
- Found to be safe and tolerable when applied to cervix
- One small Austrian RCT showed significant regression (73% vs 39%) and remission (47% vs 14%) of CIN 2,3 lesions with imiquimod vs. placebo

Pachman et al., 2012; Grimm et al., 2012
Specific Aims

- **Aim 1**: To determine the therapeutic effect of a “prime and pull” strategy in women with high grade cervical lesions using HPV vaccine with a topical immune modulator, imiquimod.

- **Aim 2**: To evaluate local and systemic immune responses in women treated with “prime and pull” strategy.
Study Design

**Women with high-grade cervical intraepithelial neoplasia (CIN 2/3)**

Patients must have histologically-confirmed pre-invasive HPV lesion(s), cervical intraepithelial neoplasia grades 2 or 3 (CIN 2/3), as determined by colposcopy-guided cervical biopsies

Randomized

- Control (observation)
- Imiquimod (16 week course)
- HPV vaccine + Imiquimod (16 week course)

Primary Outcome Assessment (Weeks 20-24)
Study Protocol Snapshot

- Imiquimod self application

- Vaccine

- Booster (if applicable)

- Baseline colpo
  Baseline biopsy

- Blood draw
  High risk HPV test
  HPV 16/18 genotype
  Cytobrush
  Urine pregnancy test

- Colposcopy
  Blood draw
  Cytobrush
  (Biopsy if necessary)
  Urine pregnancy test

- 3-6 Months after final biopsy (if regression):
  Pap
  High risk HPV test

- Colposcopy
  Biopsy
  Pap
  Blood draw
  High risk HPV test
  HPV 16/18 genotype
  Cytobrush
  Urine pregnancy test

- 3-6 Months after final biopsy (if regression):
  Pap
  High risk HPV test

2. Using HPV testing for Cervical Cancer Screening. Educate the Educators Module 1, American Society for Colposcopy and Cervical Pathology, 2016.


Video