Anal squamous precancerous lesions: LAST 2013 terminology – role of high resolution anoscopy and cytology

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Anal squamous precancerous lesions

• Some anal cancer statistics: a parallel between cervical and anal cancer
• The transitional / transformation zones
• Epidemiology of HPV-associated anal diseases
• Precursor lesions
  – Definitions, classifications
  – Screening
Some anal cancer statistics

- Incidence rates (USA)
  - Women 1.8 / 100 000
  - Men 1.4 / 100 000
- Median age at diagnosis: 60 yrs (90% after age 45)
- Median age of death: 65 yrs
- Overall: 1 in 600 people
- New cases in 2011 (USA): 5870 (770 deaths)
- Significant increase 2003-2007 (SEER)

www.cancer.org; http://seer.cancer.org

Anal cancer rates by birth cohort

Age trends come from
- sexual revolution
- HIV epidemic

Simpson JAD et al. BMJ 2011
Anal cancer and cervical cancer

- Cervical cancer (USA)
  - Prior to Pap screening: 40 - 50 / 100 000
  - Currently: 8 / 100 000

- Anal cancer (USA)
  - Women, general population: 1.8 / 100 000
  - HIV neg MSM: 35 / 100 000
  - HIV pos MSM: > 70 / 100 000

www.cancer.org ; http://seer.cancer.org

Anal cancer and cervical cancer

- Common risk factors
  - sexual intercourse: vaginal / anal
  - HPV infection: high-risk HPV (16, 18)
  - > 90% anal cancer are HPV related, especially HPV16

- Anatomic commonality
  - « Transition / Transformation » zone
  - regions with active squamous metaplasia
  - vulnerable to HR-HPV

- Morphologic similarity
  - Precursor lesions: HSIL/LSIL in CIN and AIN
  - Cancer: SCC
ATZ

- Morphologically analogous to the cervical TZ
- Transition or transformation?
- Region of squamous metaplasia
- « Immature » metaplasia
  - at squamo-columnar junction
  - Susceptible to oncogenic HPV

From Histology for Pathologists

Transition zones: an elective place for early neoplasia?

Review article
Adrian J. McNairn
Geraldine Guasch
Division of Developmental Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH

Epithelial transition zones: merging microenvironments, niches, and cellular transformation
**Evolution of the cervical squamocolumnar junction**

1. Focal induction of p63/keratin 5
2. Emergence of reserve cells (positive for SC junction-specific biomarkers)
3. Proliferation and squamous differentiation of reserve cells
4. Squamous metaplasia (negative for SC junction-specific biomarkers)

_Herf et al. J Pathol 2013_

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**Opinion**

_Trends in Microbiology_ March 2011, Vol. 19, No. 3

**Figure 1.** Anatomical and histological views of cervical and anal squamocolumnar junctions. In response to hormone-induced vaginal pH acidification and trauma at ectocervical and anal glandular epithelia are replaced by a squamous epithelium. These metaplastic conversions can be incomplete (immature) or complete (mature), depending on the persistence of the native epithelium.
Expression of inflammation markers
- Tenascin C
- Keratin 17

Runck LA et al. Cell cycle 2010

Transgenic mouse model of ATZ development: Putative anal stem cell population
Methods of screening of anal HPV-related precancerous lesions

- Anal cytology
- Digital examination « DARE » (mostly for invasive cancer)
- High-resolution anoscopy « HRA »
- HRA directed biopsies
- They all have their equivalent in cervical pathology
Anal cancer (SCC): who is at risk?

- MSM
- HIV
- Other causes of immunosuppression
  - Solid organ transplant
  - ? Other: chronic steroid therapy, cancer chemotherapy
- Women with HSIL / Cancer
  - Multifocal HPV-related disease
  - Vulvar / perianal > cervical

Prevalence of anal HPV in MSM
Population-based data

Chin-Hong et al. Ann Int Med 2008;149:300
Prevalence of AIN in MSM
Population-based data

Chin-Hong et al. Ann Int Med 2008;149:300

Anal and cervical HPV infection and HIV infection in women

Risk factors for anal HPV in HIV pos:
- Lower CD4
- Cervical HPV
- Non hispanic white
- NO relation with receptive anal intercourse

Anal SIL in women

Risk factors for abnormal cytology in HIV positive:
- Lower CD4
- High HIV RNA

Holly et al. JNCI 2001;93:843

Relative risk of cancer
US AIDS-Cancer Match Registry

Frisch et al. JNCI 2000;92:1500
Relative risk of cancer US transplant recipients

<table>
<thead>
<tr>
<th>Cancer site*</th>
<th>Observed cases</th>
<th>Expected cases</th>
<th>SIR²</th>
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<tbody>
<tr>
<td>Infection-related malignancies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>1504</td>
<td>199.4</td>
<td>7.54</td>
</tr>
<tr>
<td>Nodal NHL</td>
<td>831</td>
<td>136.6</td>
<td>6.08</td>
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<tr>
<td>Extranodal NHL</td>
<td>673</td>
<td>62.8</td>
<td>10.72</td>
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<tr>
<td>Liver</td>
<td>930</td>
<td>80.5</td>
<td>11.56</td>
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<tr>
<td>Stomach</td>
<td>152</td>
<td>90.9</td>
<td>1.67</td>
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<tr>
<td>Kaposi sarcoma</td>
<td>120</td>
<td>2.0</td>
<td>61.46</td>
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<tr>
<td>Oropharynx including tonsil</td>
<td>106</td>
<td>52.8</td>
<td>2.01</td>
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<tr>
<td><strong>Anal</strong></td>
<td>90</td>
<td>15.4</td>
<td>5.84</td>
</tr>
<tr>
<td><strong>Hodgkin lymphoma</strong></td>
<td>85</td>
<td>23.7</td>
<td>3.58</td>
</tr>
<tr>
<td><strong>Vulva</strong></td>
<td>58</td>
<td>7.6</td>
<td>7.62</td>
</tr>
<tr>
<td><strong>Cervix</strong></td>
<td>45</td>
<td>43.6</td>
<td>1.03</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22</td>
<td>5.3</td>
<td>4.13</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>8</td>
<td>8.3</td>
<td>0.96</td>
</tr>
<tr>
<td>Vagina</td>
<td>7</td>
<td>3.0</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Engels et al JAMA 2011;306:1891

Cancer incidence in women with and without a history of CIN3

Edgren, Sparen, Lancet Oncol 2007;8:311
Anal cancer: Screening guidelines
USA

• No formal screening guidelines.
• CDC: Acknowledges that some experts recommend anal cytology screening for HIV+ men and women.
• ACS: Anal cytology, sometimes called the anal Pap test, may be useful in early diagnosis of anal cancer and precancer (called AIN). Some doctors already recommend this test for people at high risk for anal cancer, such as those who are HIV positive.
• New-York State Department of Public Health AIDS Institute: Clinicians should obtain anal cytology at baseline and annually in the following HIV infected populations:
  – Men who have sex with men
  – Any patient with a history of anogenital condylomas
  – Women with abnormal cervical and/or vulvar histology

Anal cancer: Screening guidelines
France – SFED 2014

• No formal screening guidelines.
• Screening is recommended in high-risk populations.
  – HIV+ men who have sex with men
  – HIV+ women with a history of anogenital condylomas and/or dysplasia and/or cancer
• Screening consists in
  – Clinical examination
  – DARE
  – Standard anoscopy
• Anal cytology and high resolution anoscopy under evaluation
Anal cancer: Screening guidelines
France – ANRS “rapport Yeni” 2010

• Screening of AIN by cytology is promising, but still insufficiently used on large scale
• In reference centres, performances similar to cervical screening
• Gold standard still biopsies (when abnormal cytology)
• Proctologists and pathologists have to become familiar with cytological sampling and interpretation
• Future screening should be annual cytology, and if abnormal, HRA.

Anal cytology - Technique

• Moistened Dacron swabs
• Inserted into anal canal until resistance is met
  – Above anal verge to distal rectum
• Rotate / apply pressure to walls of canal while removing sampling device
• Liquid-based cytology or direct smears
Anal cytology - Goal

- Sample entire canal
- Anal transitionnal zone
  - Analogous to cervical TZ
  - Squamous metaplasia
- Non-keratinized squamous mucosa
- Keratinized squamous mucosa
- Use Bethesda System terminology
- Diagnostic criteria similar to Gyn cytology
- Guidelines for specimen adequacy

Anal cytology – Specimen adequacy

- Liquid vs conventional pap
  - Better cell preservation
  - ↑ cellular harvest
  - ↓ bacteria / fecal contamination
  - ↓ mechanical / air-dry artifacts
- Minimum cellularity
  - 2000-3000 nucleated squamous cells
- ThinPrep (20 mm)
  - 1 to 2 nucleated cells / hpf
- SurePath (13 mm)
  - 3 to 6 nucleated cells / hpf
### Anal cytology – Normal components

- **Transition zone components**
  - Rectal columnar cells (not required)
  - Squamous metaplasia
- **Nucleated squamous cells**
- **Anucleate squames**
- **You may find bugs (herpes, candida, worms)**

### 2001 Bethesda System

#### Epithelial cell abnormalities

- **Squamous cell abnormalities**
  - Atypical squamous cells
    - of undetermined significance (ASC-US)
    - cannot exclude HSIL (ASC-H)
  - Low-grade SIL (LSIL)
  - High-grade SIL (HSIL)
  - Squamous cell carcinoma
- **(Glandular cell abnormalities)**
Low-grade lesions

- Caused by both high-risk and low-risk viral types
- Most of these will spontaneously regress, if immunocompetent
- Productive HPV infection
- In general, close clinical observation without treatment is recommended (unless symptomatic)

<table>
<thead>
<tr>
<th>LAST proposal</th>
<th>Bethesda terminology</th>
<th>AIN terminology</th>
<th>Dysplasia terminology</th>
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</thead>
<tbody>
<tr>
<td>LSL / AIN1</td>
<td>LSL</td>
<td>AIN1</td>
<td>Mild dysplasia</td>
</tr>
<tr>
<td>HSIL / AIN2 - AIN3</td>
<td>HSIL</td>
<td>AIN 2</td>
<td>Moderate dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AIN 3</td>
<td>Severe dysplasia</td>
</tr>
</tbody>
</table>

High-grade lesions

- Caused by high-risk viral types
- Most of these will persist or progress
- With time integrated HPV infection
- Goal: treat precancer before it develops into cancer

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<td></td>
<td></td>
<td>AIN 3</td>
<td>Severe dysplasia</td>
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Carcinoma in situ
Anal squamous cell carcinoma

- Invasive cancer (possible)
- Associated with high-grade lesions

- No defined counterpart to cervical microinvasion
- T1 = 2 cm or less
- Not subdivided
- LAST: SISSCA

Early detection makes a difference

<table>
<thead>
<tr>
<th>Stage at diagnosis</th>
<th>Stage distribution</th>
<th>5-year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localized (confined to primary site)</td>
<td>50%</td>
<td>80%</td>
</tr>
<tr>
<td>Regional (spread to regional lymph nodes)</td>
<td>29%</td>
<td>60%</td>
</tr>
<tr>
<td>Distant (cancer has metastasized)</td>
<td>12%</td>
<td>30%</td>
</tr>
<tr>
<td>Unknown (unstaged)</td>
<td>9%</td>
<td>56%</td>
</tr>
</tbody>
</table>

Seer.cancer.gov
Anal cytology
Sensitivity and specificity

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity *</th>
<th>Specificity *</th>
</tr>
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<tbody>
<tr>
<td>HIV+</td>
<td>81%</td>
<td>63%</td>
</tr>
<tr>
<td>HIV -</td>
<td>50%</td>
<td>92%</td>
</tr>
</tbody>
</table>

* Includes ASCUS. Conventional smears

Palefky et al. J AIDS & Hum Retrovir 1997;14:415

Anal cytology / Histology

- Sample adequacy issue
- Anal cytology often under-represents grade of disease
- Anal cytology is complementary to:
  - HRA and
  - Histology and
  - Digital anorectal examination
- « Gold » standard: HRA-guided biopsy
- Link screening to treatment
- Anal HPV testing? No approved HPV test for anus, mixed reports of usefulness and cost-effectiveness
High resolution anoscopy
«Anal colposcopy »
Dr Isabelle Etienney

- Anal colposcopy
  - Acetowhite lesions
  - Contour changes
  - Vascular changes
HRA guided biopsies
Some answers to current questions

• Positive
  – The incidence of AIN and anal cancer is high among HIV + women and MSM (both HIV – and HIV +)
  – HAART has limited effect on HPV-related neoplasia
  – The incidence of anal cancer will probably continue to rise among HIV + MSM
• Positive
  – At-risk men and women should be screened for anal cancer with digital exam, as early detection of anal cancer has real benefits
  – At-risk men and women should be considered for screening and treatment of AIN (treatment is improving)
  – HPV vaccines have the potential to prevent anal HPV infection and ultimately, anal cancer.
Still many current unknowns

- Who should we really screen? (HIV+, men, women, transplant recipients, women with HSIL, men with perianal condylomas?)
- Will screening and treatment of AIN lower the incidence of anal cancer?
- Is effective treatment of AIN possible?

Anal precancerous lesions: a confusing terminology...

- Dysplasia
- ASCUS
- AIN
- ACIN
- Bowen
- m – M - S
- LSIL
- Carcinoma in situ
- HSIL
- ASCH
- PSIN
- ASIL
- Leucoplasia
Anal precancerous lesions: the LAST terminology (Darragh T. APLM 2012)

**WHO 2010**

*In anal canal chapter, it is said:*

- “Precancerous intraepithelial neoplasia of the ATZ has also been named dysplasia, carcinoma *in situ*, ASIL. The same lesion in the perianal skin is usually called Bowen disease”.
- AJCC recommends to use LSIL and HSIL.
- WHO recommends « AIN ».
**Anal precancerous lesions in the WHO 2010 classification**

*In the table on classification of anal canal cancers*

- **WHO classification**
  - Precancerous lesions
    - AIN (dysplasia), low grade
    - AIN (dysplasia), high grade
    - Bowen disease (PSIN)

- **TNM classification**
  - Tis: *in situ* carcinoma, Bowen disease, HSIL, AIN II-III (*no mention of microinvasive carcinoma, another subject...*)

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**Terminology**

<table>
<thead>
<tr>
<th>Year</th>
<th>Procedure</th>
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<tbody>
<tr>
<td>1900</td>
<td>Hysterectomy</td>
</tr>
<tr>
<td>1932</td>
<td>Exclusion CKC LEEP</td>
</tr>
<tr>
<td>1953</td>
<td>Ablation Cryo Laser</td>
</tr>
<tr>
<td>1967</td>
<td>Option to Rx or to follow</td>
</tr>
<tr>
<td>1980s</td>
<td>No Rx</td>
</tr>
</tbody>
</table>

*Darragh et al, Arch Pathol Lab Med 2012*
**Anal squamous precancerous lesions through the ages…**

- Before 1960: as in the skin
- 1962: distinction anus / perianus
- 1971: Bowen disease has a viral origin
- 1981: Anal dysplasia (Fenger)
- 1986: Anal intraepithelial neoplasia (AIN) 1/2/3 (Fenger)
- 1995: HPV in anal cancer (IARC)
- 1996: Anal squamous intraepithelial lesion (SIL)
  - Low grade (LSIL): AIN 1
  - High grade (HSIL): AIN 2 + AIN 3
- 2012: LAST project (*Darragh T et al, Arch Pathol Lab Med*)
« LAST » recommandations
Standardisation of terminology of squamous anogenital lesions associated to HPV

• CAP and American Society of Colposcopy and Cervical Pathology
• Histopathological and cytopathological terminology
  – The nomenclature has to be the same for the entire lower anogenital tract.
  – It has to be a 2-tiered system.
  – Recommended terminology : HSIL / LSIL, that may be subcategorized with « IN » (1/2/3) for histo.

  Darragh TM et al. Arch Pathol Lab Med 2012

« LAST » recommandations
Standardisation of terminology of squamous anogenital lesions associated to HPV

• Terminology for histo joins cytology, « IN » mention is used to signify it is histopathology
• LSIL diagnostic criteria:
  – Proliferation with nuclear atypia, preserved maturation, mitoses lower 1/3, And/or
  – HPV cytopathogenic effect, no signs of high-grade
• HSIL diagnostic criteria:
  – Proliferation with nuclear atypia, loss of maturation, mitosis over the lower 1/3.

  Darragh TM et al. Arch Pathol Lab Med 2012
Special situations:
- Abnormal mitoses in the lower 1/3 : HSIL? \( \rightarrow \) p16…
- "Thin SIL"
- Keratinizing SIL (peri-anal skin)
- Extension to cervical (and anal) glands
- Condyloma accuminata : LSIL by definition
- Bowenoid Papulosis : along clinical context, you cannot ascertain on small biopsies

Recommendations on "SISCCA" (superficially invasive squamous cell carcinoma), including anus

Darragh TM et al. Arch Pathol Lab Med 2012
« Micro-invasion »

- No consensual definition in the anus
- Superficially invasive squamous cell carcinoma (LAST) (SISCA): Total excision, size of infiltrative component ≤ 7mm, depth ≤ 3mm.
Anal SISCA - Survival free of disease
Arana et al. In press

Courbe de survie sans cancer des 17 cas de CE "micro-invasifs"

Courbe de survie des petits CE T1N0M0 traités par radiothérapie


« LAST » recommandations
Standardisation of terminology of squamous anogenital lesions associated to HPV

- Major difference with other histopathological classifications of digestive precancerous lesions (Riddell, Vienne) : no category « indefinite, could be, I don’t know… »
- However, there are HSIL « mimickers » :
  - Immature squamous metaplasia
  - Atrophy
  - Repair
  - Tangential sections
- The solution: immuno p16 !

Darragh TM et al. Arch Pathol Lab Med 2012
"LAST" recommandations p16 IHC

- Review of the literature on biomarkers: 2291 articles, 72 selected (!), 53 on p16
- p16, Ki67, ProEx C, L1, ARN HPV 16/18, Telomerase, HPV genotypage: only p16 is recommended (eventually with Ki67, ProEx C)
- p16+: intense and diffuse, nuclear (+/- cytoplasmic), at least 1/3 of epithelium.

Darragh TM et al. Arch Pathol Lab Med 2012
• You may HPV in a « normal appearing » epithelium
• p16 is sensible and specific for AIN 2-3 (HSIL)

« LAST » recommandations
p16 IHC

• Diagnostic doubt between IN 2 or 3 and « mimicker » of HSIL
• Diagnosis of IN 2 :
  – p16 + → precancer (HSIL)
  – p16 - → LSIL, or lesion unrelated to HPV
• Discordance between pathologists, at least one says > IN 2
• Diagnosis ≤ IN 1, in a patient at risk for HSIL, i.e. HSIL on cytology, ASC-H, ASC-US/HPV16+
• Not indicated in other cases (≤ IN1 and ≥ IN3)
• This represents less than 20% of cervical biopsies (anus??)

Darragh TM et al. Arch Pathol Lab Med 2012
In conclusion regarding anal squamous precancerous lesions

The role of precancerous lesions in the development of anal SCC is recognized, but their natural history is not well established.

Their classification and diagnostic criteria are becoming consensual, identical to those in use for the uterine cervix (LAST).

The respective role of cytology and histology is still under evaluation, depending on which population.

Still the same problems (sampling, natural history, reproducibility…) as for other precancerous lesions in the GI tract. p16 is helpful in histology (and probably in cytology).
AIN on hemorrhoidectomy specimens

- 1 to 4% of « unremarkable » cases (3.2% in a personal prospective series of 3000 haemorrhoidectomy – Etienne ey al, in press)
- No predictive factors (F > M)
- Localised at least on ATZ, extends to squamous and/or glandular mucosa
- Usually high grade (III)
- Frequent signs of HPV infection
- Look for microinvasion (almost never present)
- Treatment : hemorrhoidectomy then surveillance