INITIATION OF AN ANAL CANCER SCREENING IN HIV INFECTED MSM:
- RESULTS OF CYTOLOGY, BIOPSY AND DETERMINATION OF RISK FACTORS
- PRACTICAL ISSUES

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Introduction

- Incidence of anal cancer is increasing since 20-30 years
- Anal cancer have similitude with cervical cancer:
  - associated with HPV
  - existence of squamous intra-epithelial lesions as precursor of invasive cancer
- Longer life expectancy and aging of HIV infected persons: \( \uparrow \) cancers
- Risk of anal cancer is higher in HIV+, especially in MSM patients (Silverberg et al. Clin Infect Dis 2012)
Epidemiology of anal cancer in HIV-positive patients

13 cohorts from North America 1996-2007

- 34,000 HIV-infected patients
  - 55% MSM
  - 19% heterosexual Men
  - 26% Women
  - mostly Caucasian
  - mostly Black and heterosexual HIV transmission

- 114,260 HIV-negative patients

### Table

<table>
<thead>
<tr>
<th>Calendar Era</th>
<th>Rate Ratio (95% CI)</th>
<th>Standardized Incidence Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSM</td>
<td>Other Men</td>
</tr>
<tr>
<td>1996–1999</td>
<td>60.8 (28.3–130.3)</td>
<td>18.2 (3.9–85.0)</td>
</tr>
<tr>
<td>2000–2003</td>
<td>100.8 (51.7–196.5)</td>
<td>27.3 (9.1–81.8)</td>
</tr>
<tr>
<td>2004–2007</td>
<td>78.8 (40.8–152.1)</td>
<td>31.9 (11.9–85.4)</td>
</tr>
</tbody>
</table>

Silverberg M and al. CID 2012
Introduction (2)

- Younger age in HIV+: 40 y vs 60 y in HIV neg
- Antiretroviral therapy (ARV) not associated with a ↓ risk of anal cancer (controversial)
Prevalence of anal HPV in HIV+

- MSM: 85-95%
- Women: 76-90%
- Heterosexual men: 60%
- IVDU: 46%

Integration of HPV into the DNA of the infected host cell is commonly associated with high-risk oncogenic HPV types, and is linked to the activity of E6 and E7 protein.
In benign HPV-associated skin lesions, the HPV virus maintains its genome as episomes at low copy numbers in the basal cells of the epithelium separate from the host cell DNA.
Integration of HPV into the DNA of the infected host cell is commonly associated with high-risk oncogenic HPV types and is considered an important step in tumor progression.
Human defenses against Papillomavirus

- No cell death
- No inflammation
- No ulcer, no exudate
- No antigen presenting cell

- HPV has immune evasion capability

- Transmission by
  - Genital contact
  - Skin contact
  - Self inoculation

- Weak antibody response in 50-90% of persons
Immune events in regression

- Regression of warts
  - Active cell-mediated immune response
  - T-lymphocytes: CD4+, Antigen experienced
  - Upregulation of interferon

- Regression of dysplastic lesions due to cellular immunity against HPV specific antigen (E6 and E7)
Interactions between HIV and HPV

• HIV infection promotes HPV infection and induced HPV lesions

• HPV infection favours acquisition of HIV infection
Interactions between HIV and HPV

- HIV infection promotes HPV infection and induced HPV lesions

- HPV infection favours acquisition of HIV infection

1. MSM (1 study): not if 1 HPV types
   Anal or cervical cytological abnormalities not associated with HIV acquisition (few studies)

2. Women

3. Heterosexual men (1 study)

Chin-Chong et al. AIDS 2009
Houlihan et al. AIDS 2012
Infection with HPV and HPV-induced lesions in HIV-positive MSM

- **HPV Prevalence:**
  - all HPV 93% (vs. 64%)
  - HR HPV 74% (vs. 37%)
  - Plateau from young to 50-60 years old

- **Prevalence HGAIN**
  - 29%
  - Risk increases with age
    - 40-49 years OR 3.09
    - >50 OR 4.78
    - Compared to <40 years

- **Incidence of HG-AIN:**
  - 8.5-15.4% patients year
  - vs. 3.3-6% patients year in HIV-neg MSM

European guidelines for treatment of HIV infected adults recommend anal cancer screening by digital rectal exam ± Pap test and anoscopy if Pap test is abnormal.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Patients</th>
<th>Procedure</th>
<th>Evidence of benefit</th>
<th>Screening interval</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>Women 50-70 yrs</td>
<td>Mammography</td>
<td>↓ breast cancer mortality</td>
<td>1-3 years</td>
<td></td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Sexually active women</td>
<td>Papanicolau test</td>
<td>↓ cervical cancer mortality</td>
<td>1-3 years</td>
<td>Target age group should include at least the age range 30 to 59 years. Longer screening interval if prior screening tests repeatedly negative</td>
</tr>
<tr>
<td>Anal cancer</td>
<td>Homosexual men</td>
<td>Digital rectal exam ± Papanicolau test</td>
<td>Unknown - advocated by some experts</td>
<td>1-3 years</td>
<td>If Pap test abnormal, anoscopy</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Persons 50-75 yrs</td>
<td>Faecal Occult Blood test</td>
<td>↓ colorectal cancer mortality</td>
<td>1-3 years</td>
<td>Benefit is marginal</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Men &gt; 50 yrs</td>
<td>Digital rectal exam ± Prostate specific test</td>
<td>Controversial</td>
<td>1-3 years</td>
<td>Pros: ↑ early diagnosis Cons: Overtreatment, no ↓</td>
</tr>
</tbody>
</table>

Anal screening in HIV patients

Optimal screening strategy?

- Swab and after HRA if abnormal swab?
- HRA directly?
- HPV testing? >90% positive
  highly sensitive but poor specificity

Salit I et al. AIDS 2010
Palefsky et al. Current Infect Dis Rep 2010
Is cytology good enough to detect AIN in HIV patients?

- Sensibility to detect AIN: 69-93% (Chiao et al. CID 2006)
- Specificity to detect AIN: 32-67%
- Anal cytology is a poor predictor of the severity of AIN lesions
- In HIV-, sensibility (50-60%), specificity (76-82%)

Table 1. Performance of anal cytology compared with high-resolution anoscopy examination findings and histology.

<table>
<thead>
<tr>
<th>Performance of anal cytology</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRA assessment</td>
<td>69% (218/315)</td>
<td>64% (171/267)</td>
<td>69% (218/314)</td>
<td>64% (171/268)</td>
</tr>
<tr>
<td>95% CI</td>
<td>64-74</td>
<td>58-70</td>
<td>64-74</td>
<td>58-70</td>
</tr>
<tr>
<td>Histology (any grade)</td>
<td>70% (182/261)</td>
<td>67% (10/15)</td>
<td>97% (182/187)</td>
<td>11% (10/89)</td>
</tr>
<tr>
<td>95% CI</td>
<td>64-75</td>
<td>38-88</td>
<td>94-99</td>
<td>85% (89/102)</td>
</tr>
<tr>
<td>Histology (high grade)</td>
<td>81% (57/70)</td>
<td>37% (76/206)</td>
<td>30% (57/187)</td>
<td>76-92</td>
</tr>
<tr>
<td>95% CI</td>
<td>0-90</td>
<td>30-44</td>
<td>24-38</td>
<td>76-92</td>
</tr>
</tbody>
</table>

Sensibility ↑ if extensive disease and if CD4 <400

Nathan et al. AIDS 2010
predictive value if repeat testing: after 2 consecutive years
(Palefsky et al. JAIDS 1997)

- PPV 38% to 78%
- NPV 46% to 79%
Is cytology good enough to detect AIN in HIV patients?

- 400 HIV+ MSM
- Anal cytology, anal HPV testing and HRA with biopsies
- HPV 93% (HPV16 38%, HPV18 20%)
- Cytology abnormal 67%, 25% AIN2+

Any abnormality on anal cytology to detect AIN2+:

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>84%</td>
<td>39%</td>
</tr>
<tr>
<td>NPV</td>
<td>88%</td>
<td>PPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31%</td>
</tr>
</tbody>
</table>

Salit et al. AIDS 2010
HRA in first line screening?

- **Pro:**
  - Histology considered as gold standard (but controversy exist)
  - A negative swab had not a very good negative predictive value (if high prevalence of HGAIN) but better if repeated

- **Con:**
  - Cost
  - Cytology can give additional information, ex: HSIL with normal biopsy = HRA to be repeat.
  - Availability of HRA
  - Willingness of patients to have this procedure
Anal screening in HIV patients

- Cytology
  - Normal
    - Repeat in 12 months (HIV+)
    - Repeat in 2–3 years (HIV–)
  - ASC-US
  - LSIL
    - Anoscopy with biopsy
      - No lesion seen
      - AIN I
        - Follow-up in 6 months or treat if minimal potential for morbidity
      - AIN II or III
        - Treat
  - HSIL (or ASC-H)

Implementing anal dysplasia screening in Brussels

- A systematic anal cancer screening in HIV+MSM was established in June 2011 in the AIDS reference centre of university Saint-Pierre hospital in Brussels.

- Cohort Saint-Pierre:
  - 2750 patients
  - 925 MSM = 33%
Methods (1)

- Anal cytology was systematically performed in HIV+MSM. Dacron swab was collected by the clinician or self-collected by the patient and then was placed in liquid cytology media.

- If anal cytology was abnormal (even ASCUS), **high resolution anoscopy (HRA)** with biopsy was performed by the same proctologist.

- Anal cytology was categorized according to the modified Bethesda System (as cervical cytology):
  - HSIL= high-grade squamous intraepithelial lesions
  - LSIL= low grade squamous intraepithelial lesions
  - ASC-US= atypical squamous cells of undetermined significance
  - ASC-H = atypical squamous cells, cannot rule out a high grade lesion.

- Results of biopsies (histology) were classified as normal, or according to severity: anal intraepithelial neoplasia (AIN) 1, 2 or 3 (the cancer precursor)
Methods (2)

- All samples were assessed by the same expert cytopathologist (F. Feoli)
- First 250 cytology were reviewed by an other cytopathologist (G. Negri, Italia) for quality control
- Data were collected prospectively.
Results (1): patients

- 353 HIV+ MSM screened by anal smears between June 2011 and May 2012.
- 90% Caucasians
- Median age 44 years (range 22-71 years)
- 83% on ARV and 74% with viral load (VL) <20 cp/ml
- Median CD4 632/µl
- Nadir CD4<200 33%  CD4<100 17%
- Median HIV duration 8 years (IQR: 4-18)
- Median ARV duration 7 years (IQR: 2-15)

IQR: interquartile range
Results (2): cytology

- Thirty-three (9.3%) swabs were excluded because of poor quality.
- **On the 320 analyzed smears, 147 (46%) were abnormal.**

In patients with normal cytology (univariate analysis):

- HIV viral load more frequently undetectable (82% vs 64%, p=0.0003)
- Median duration of HAART longer (111 vs 61 months, p= 0.0145)
- No correlation was found for age, current or nadir CD4
### Comparison with others studies

<table>
<thead>
<tr>
<th>Number of participants (MSM)</th>
<th>Normal %</th>
<th>HSIL %</th>
<th>LSIL %</th>
<th>ASC-US %</th>
<th>ASC-H %</th>
<th>Risk factors for abnormal cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study, Brussels 2012</td>
<td>54</td>
<td>3</td>
<td>24</td>
<td>16</td>
<td>3</td>
<td>VL detectable Time with ART shorter</td>
</tr>
<tr>
<td>SUN, US, Conley et al. JID 2010</td>
<td>44</td>
<td>9</td>
<td>31</td>
<td>14</td>
<td>3</td>
<td>Nadir CD4&lt;50 Current CD4 &lt;500 No of high risk HPV</td>
</tr>
<tr>
<td>Canada, Salit et al. AIDS 2010</td>
<td>33</td>
<td>12</td>
<td>43</td>
<td>12</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Canada, De Pokomandby CID 2011</td>
<td>33</td>
<td>6</td>
<td>26</td>
<td>33</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
Results (3): HRA and biopsies

For this analysis, high grade AIN (2 and 3) were put together (AIN 2+).
Swab and biopsies

<table>
<thead>
<tr>
<th>SWAB</th>
<th>Biopsy</th>
<th>Normal</th>
<th>AIN 1</th>
<th>AIN 2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ASCU</td>
<td>3</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>ASCH</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>LSIL</td>
<td>1</td>
<td>23</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>36</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

Among patients with AIN 2+, cytology didn't show high-grade lesions in 81% of the cases (8 (24%) ASC-US, 3 (9%) ASC-H, 19 (57%) LSIL, 3 (9%) HSIL).
When patients with normal cytology or normal biopsy and patients with AIN 2+ were compared: the only significant risk factor found for AIN 2+ was a nadir CD4 <100/µl (32% of the patients with AIN 2+ vs 14% in patients with normal smear, p=0.0119).

No correlation for CV<50, HAART duration.
Conclusion of this first year of screening

- Anal precancerous lesions are frequent and at different stages.
- Among patients with abnormal cytology, 87% had abnormal biopsy including half high grade AIN.
- A normal cytology was associated with an undetectable VL and a longer duration of cART (argument for early initiation of ARV).
- Risk factor for AIN2+ was a nadir CD4<100/µl.
- Screening is the only way to detect anal dysplasia and should be performed systematically in HIV+MSM.

BUT....
Anal screening should be implemented in MSM HIV+ patients …… but questions remain

In real life, not so easy….?
Implementing anal screening

- Technique related
  - Self collected cytology
  - Training

- Operator related

- Side effects:
  - Immediate or delayed
  - Pain, bleeding and/or infection
  - Local Anesthesia (cream)
  - Small multiple biopsies
  - Prescribe medication
  - Inform the patient
All MSM not screened for anal dysplasia

- 38% of MSM had anal swab during one year
  - Some patients directly to gastroenterology for other reason and HRA was performed but the others?
  - Patients without swab
    - were less treated (73% vs 83%, p=0.017) and when treated for a shorter period (65 vs 93 months, p=0.02)
    - Had a shorter duration of HIV infection (75 vs 101 months, p<0.0001)

- Patients with abnormal anal swab and no biopsies. In investigation...

Cohort Saint-Pierre Hospital
1 missed cancer: men 50 y

- 08/2011: normal swab but symptoms of rectitis/anitis
  - LGV but persistent complaint after treatment
  - anoscopy: stenosis at 4 cm
- First biopsy 09/11: AIN 1
- Second biopsy 10/11: AIN 2 + microinvasif carcinoma

Cohort Saint-Pierre Hospital
How to treat AIN?

- Total excision not possible for AIN
- Natural evolution not well known (Marks et al. JAIDS 2012)
  - « Watch and wait »? BUT
    - Rate of progression of HGAIN to anal carcinoma estimated to be 8-13% (retrospective studies)
    - Rate of progression to anal carcinoma in series on HGAIN treatment: 0%-1.2%
- Treatment of anal carcinoma associated with significant morbidity

- Few studies on treatments

⇒ TREAT HGAIN
Treatment of high grade lesions

- **Ablative techniques**
  - CO2 laser
  - Laser, electrocautery, infrared coagulation, surgery

- **Topical treatment**
  - Trichloro acetic acid: efficient in low grade, limited diseases
  - Imiquimod
  - 5 Fluoro-uracil cream

- **Therapeutic vaccine**: study ongoing

To reduce extent of the disease, on study. Jay et al.
Imiquimod for HGAIN? One study RCT

For multifocal anal lesions and as adjunction to ablative therapy (reduce extensive surgery)

Fox et al. AIDS 2010
## Treatment of AIN

- 150 HIV+ MSM, Amsterdam, 57% HGAIN

<table>
<thead>
<tr>
<th></th>
<th>Imiquimod (3x/w)</th>
<th>5FU (2x/w)</th>
<th>Electrocautery (1x/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response rate</td>
<td>39%</td>
<td>29%</td>
<td>48%</td>
</tr>
<tr>
<td>Complete response</td>
<td>26%</td>
<td>17%</td>
<td>41%</td>
</tr>
<tr>
<td>Recurrence after 6 months</td>
<td>25%</td>
<td>57%</td>
<td>17%</td>
</tr>
<tr>
<td>Side effects</td>
<td>43%</td>
<td>27%</td>
<td>18%</td>
</tr>
</tbody>
</table>

Imiquimod 90% complete response if perianal lesion

Treatment of AIN

- **Infrared coagulation:**
  - Cure rates 81% HIV-  
  - 72% HIV+  
  - But recurrence!

- **Electrocautery ablation (ECA)** (132 HIV+, 100 HIV-, proximal lesion to the anal verge, not too extensive, in-office treatment)
  - Cure rates after first ECA 85% HIV-  
  - 75% HIV+
  - Recurrence at 1 year: 53% HIV-, 61% HIV+ (more recurrence if >1 HGAiN). Recurrence in non treated area (ECA does not eradicate HPV…)

Marks D et al. JAIDS 2012
Regression of SIL with ART?

Longitudinal study: Anal cytology, histology, HPV before ART, at 12 and 24 months after

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=76)</th>
<th>12 months (n=68)</th>
<th>24 months (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSIL</td>
<td>36% (27/76)</td>
<td>34% (23/68)</td>
<td>33% (23/69)</td>
</tr>
<tr>
<td>HSIL</td>
<td>9% (7/76)</td>
<td>10% (15/68)</td>
<td>9% (6/69)</td>
</tr>
<tr>
<td>Total</td>
<td>59% (45/76)</td>
<td>59% (40/68)</td>
<td>52% (36/69)</td>
</tr>
</tbody>
</table>

Regression of the severity of the lesions (without treatment) in 44%
Occurrence of a new lesion between baseline and 24 months in 37%

No regression of SIL with ART in this study but
- cytology and no histology (and not a good correlation)
- duration of ART to short to detect an effect?

Piketty et al. AIDS 2013
Treatment of anal dysplasia in HIV-infected patients in Saint-Pierre Hospital

- **AIN1:**
  - close follow up (6 months)
  - no treatment except if condyloma
  - *Indication of cART?*

- **AIN2+:** treat
  - Topical treatment: Imiquimod
    - In lesions < 1cm$^2$
    - In multifocal lesions as adjunctive to ablation
  - electrocoagulation
  - *Indication of cART?*
Follow up after treatment

- AIN 2+: 1x/3 months with HRA
- AIN 1: 1x/6 months

Follow up if cytology normal

- Repeat anal swab 1x/year (no guidelines, expert opinion)
Therapeutic vaccine

• **Vulvar cancer**  
  Kenter. *NEJM* 2009  
  - 30 HIV-negative women with HPV-16 VIN3  
  - 3-4 injections (2.8 ml) mix of long peptide from HPV-16 E6 and E7  
  - Swelling 100% Fever 64%  
  - At months 12, 15/19 had clinical response (CR=9), +1 carcinoma  
  - At months 24, the 9 CR maintained, +1 carcinoma in situ

• **Anal cancer**  
  Anderson. *JAIDS* 2009  
  - Randomised, double-blind, placebo- controlled: 35 MSM HIV+ (CD4=627)  
  - 3 doses 0.5 ml at Day 1, 14/30, 70/90 HPV-16 E6E7  
  - Safety: moderate to severe short term reaction 5/35 had transient VL  
  - Strong and durable Ab response. Moderate IFN G response during 6 months  
  - Not powered to detect clinical efficacy
Prevention vaccin for young MSM?

• HPV vaccin could reduce anal cancer

• HPV vaccin could reduce HIV acquisition
  
  (Houlihan et al. AIDS 2012)
  
  – Cross protection between genotypes and new vaccin with more genotypes on study
  
  – No protection against all genotypes currently available.

• Not easy to reach young MSM before infection by HPV ➔ vaccin all young boys?
Screening of anal dysplasia in others group?

- **Women?**
  women with a history of abnormal cervical or vulvar histology

- **MSM HIV neg?** Yes if anogenital warts, others?

NewYork State Department of Health AIDS Institute guidelines
Conclusion

- Anal screening should be proposed to HIV+ MSM... and in HIV+ women with cervix or vulvar dysplasia?
- But screening as to be perform in collaboration with experienced/motivated anatomopathologist and trained gastroenterologist for HRA and treatment.
- cART should probably be initiated in patients with anal dysplasia....at least those of high grade.

- Imiquimod can be used as adjuvant treatment for multifocal anal lesion of high grade.
- Therapeutic vaccin are on study.