



RAJIV GANDHI CANCER INSTITUTE & RESEARCH CENTRE

# “ROLE OF HPV DNA TESTING IN CIN MANAGEMENT”



# Evidence-based Medicine

- ❖ Advances in HPV DNA technology, colposcopy, simple electrosurgical procedures
- ❖ New data on Epidemiology, Natural History and Treatment of CIN (CAPS >1.7 million, ALTS)
- ❖ ASCCP guidelines (2001)
  - Management of women with cytological abnormalities in cervical cancer precursors
  - Management in histological proven CIN

**A sea change in diagnosing & managing HPV & cervical disease**

## Who is “at risk”??

**Virtually any woman! But risk is enhanced if:**

- ❖ **Begin early sexual exposure; age <20**
- ❖ **Have had multiple partners**
- ❖ **Have genital human papillomavirus OR**
- ❖ **Develop other sexually transmitted infections such as genital herpes, chlamydia etc**

# Clinical Suspicion of CIN



- ❖ **Asymptomatic**
- ❖ **H/O : Excessive vaginal discharge**
- ❖ **Irregular vaginal bleeding, discharge, foul smell**
- ❖ **Post-coital bleeding**
- ❖ **Postmenopausal bleeding**
- ❖ **O/E : Cervix hypertrophied look unhealthy**
- ❖ **Hard to feel, growth may be seen**

Cancer is usually asymptomatic

the most common symptom is abnormal (ie non-menstrual) bleeding. Menstrual bleeding may be heavier and last longer.

Abnormal bleeding, particularly after the menopause

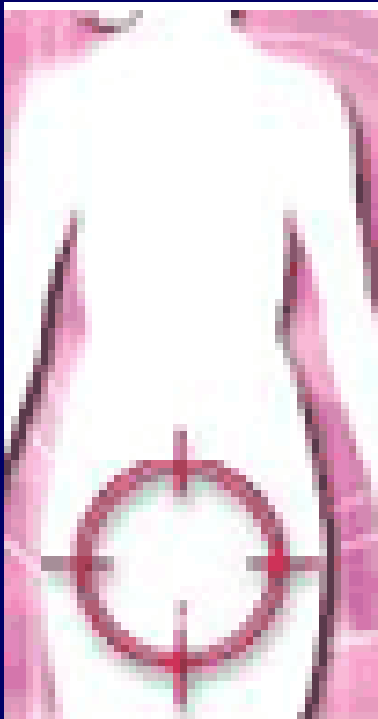
- any sort of unusual vaginal discharge
- pain in the pelvic area
- painful or difficult urination

# Classifications of Cytomorphology of Cervix

## Changing terminologies

Papanicolaou Class	WHO System	CIN	Bethesda System
Class I	Normal	Normal	Within normal limits (WNL)
Class II	Atypical	Atypical	Benign cellular changes (or) ASC-US >> ASC & ASC-H
Class III	Dysplasia	CIN	Squamous epithelial cell Abnormality
	Mild	1	Low grade SIL
	Moderate	2	
	Severe	3	
Class IV	Ca in situ		High-grade SIL
Class V	Invasive sq cell Ca	sq cell Ca	sq cell Ca
	Adeno Ca	Adeno Ca	Adeno Ca

# Genital HPV Infection



- ❖ Quite common in young sexually active women
- ❖ 70% sub clinical, 30% present as condylomas
- ❖ If untreated lesions may remain unchanged, resolve, or may progress
- ❖ Most cervical HPV infections are LGSIL : self limiting proliferations that resolve
- ❖ Some persistent infections with high risk HPVs progress to HGSIL, and to invasive carcinoma

# HPV DNA Test

- **Hybrid capture 2 HPV DNA test is semi quantitative test with clinical detection level at 5000 HPV genome/ml**
- **It uses RNA probes specific for full length genomes of oncogenic types HPV 16,18,33,35,39,45,51,52,56,58,59,68.**
- **Combined with pap test, it has 100% negative predictive value.**

# **HPV DNA Testing is not Intended as**

- ❖ A substitute for pap smear**
- ❖ For screening women below 30yrs of age**

# Interpretation of HPV DNA Test Result

- ❖ **HPV-ve & Pap -ve:** The probable risk of developing cervical cancer over next 5-15 yrs is minimal (0.2%).
- ❖ **HPV-ve & Pap +ve:** Repeat smear after 6 months colposcopy is not recommended.
- ❖ **HPV+ve & Pap -ve:** 116 fold risk of developing HSIL. Colposcopy is recommended.
- ❖ **HPV +ve/pap +ve:** these women are at higher risk (6-7% or more) of developing cancer cervix if not treated. Colposcopy is recommended to detect CIN.

# Interpretation of HPV DNA Test Result

## When colposcopy is recommended:

- ❖ If normal repeat HPV DNA test in 1-3 yrs
- ❖ If repeat HPV DNA test is negative, repeat pap test in 7 yrs
- ❖ Abnormal colposcopy detect CIN and treat it
- ❖ Borderline +ve : repeat HPV DNA test after 3yrs

# Indications for HPV DNA Testing

- ❖ ASCUS pap smear
- ❖ Resolution of disparity between cytology, colposcopy & histology
- ❖ Follow up after treatment for “Test of Cure”
- ❖ Follow up after normal colposcopy in abnormal pap smear
- ❖ Population screening as adjunct to pap smear
- ❖ In future as Primary Screening test

# Treatment of Genital HPV warts

Known to be caused by the Low Risk HPV Types

- Aim to relieve symptoms & cosmetic reasons
- Does not eradicate the virus
- Recurrence in 25-50%
- Not proven to reduce transmission of HPV
- Development of cancer cervix not influenced

# Vulvar Lesions

## Chemical Methods

- \* Podophyllotoxin 0.5%
- \* Imiquimod cream
- \* Podophyllin 20%
- \* Trichloroacetic acid
- \* Interferon
- \* 5- Fluorouracil

**Patient Applied**

**Health Care  
Provider Applied**

## Physical Methods

- \* Cryotherapy /  
Electrocautery / LASER
- \* Surgical Excision

**Trained Clinician  
Care Applied**

# Physical Methods

## CO2 LASER

- \* Useful for extensive lesions
- \* Rapid tissue healing

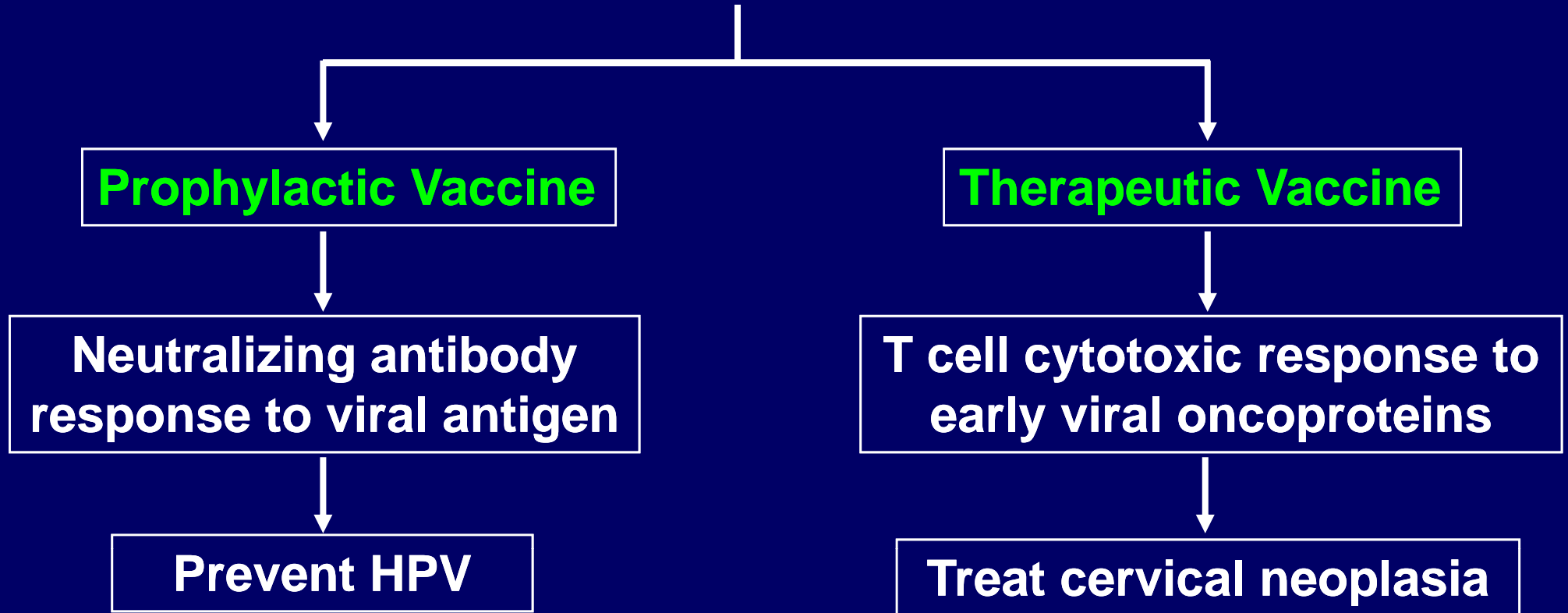
## Surgical Excision

- \* Useful for extensive lesions
- \* Useful for perianal, anal warts

# Prevention & Control of HPV

- ❖ Educate about the virus, contagious nature
- ❖ Advise about personal hygiene
- ❖ Abstinence / use of condom during treatment
- ❖ Proper follow up
- ❖ Management of sexual partners
  - Treatment & counseling if obvious lesions
  - Offer check up for other STD

# HPV DNA Typing & Vaccination

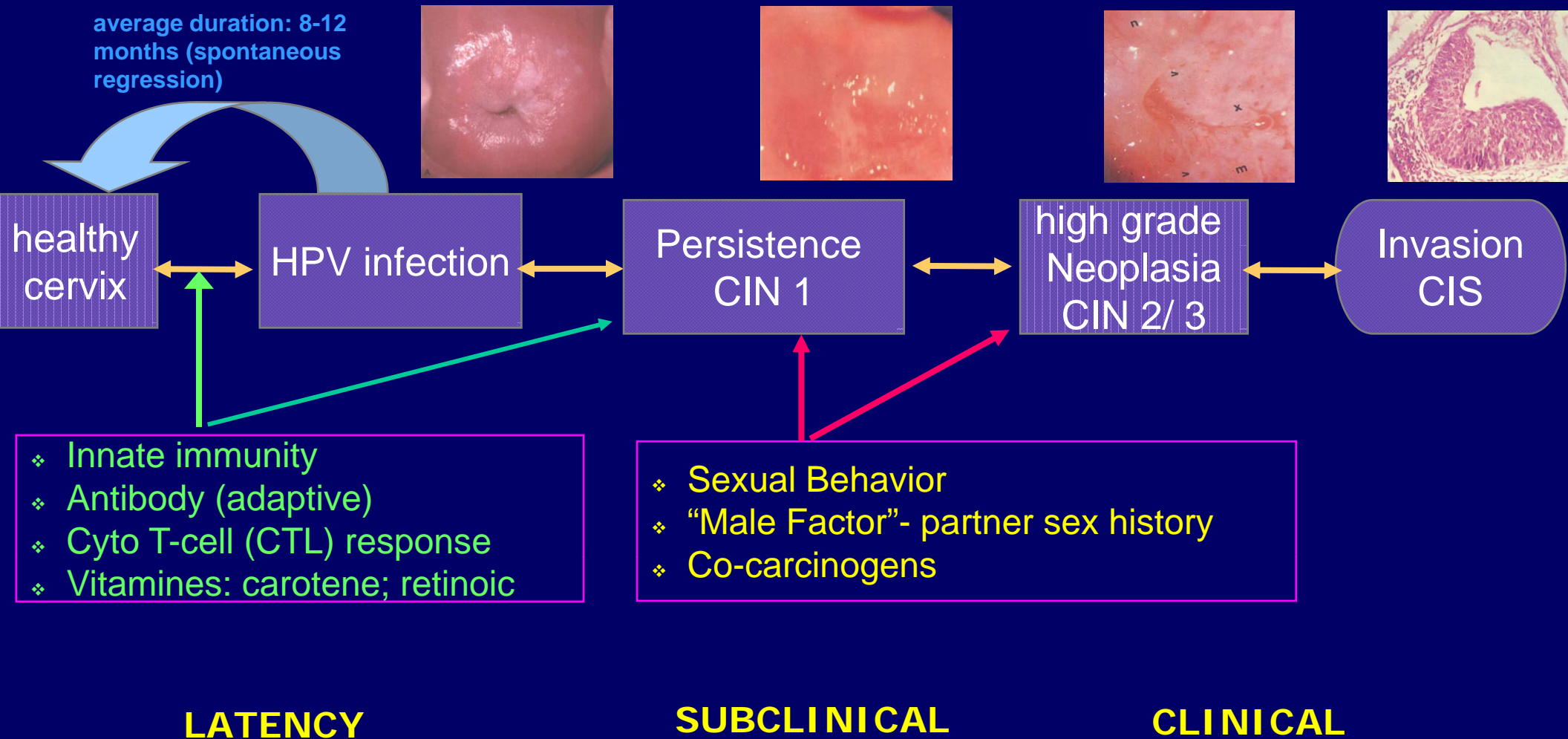


# HPV DNA Typing & Vaccination

- ❖ Merck research lab is currently conducting phase 3 trial of HPV vaccine containing 16, 18, 6 & 11.
- ❖ Another trial with HPV vaccine for type 16 (Associated with 50% Ca Cx).
- ❖ All patients tested and found negative for type 16 at the start of the study and repeated at 7 months for antibody.

<b>No. of Patients</b>	1194 with vaccine	1198 with placebo
<b>Antibodies at 7 months</b>	1510 with vaccine	6m mu/ml
<b>Incidence of persistent HPV vaccine</b>	0	3.8 per 100 women year

# Cervical Carcinogenesis: a typical model



# Management of Cervical Cytologic Abnormalities

## The 2001 Bethesda System

### **Squamous Cell Abnormalities**

- ❖ Atypical squamous cells (**ASC**)  
of undetermined significance (**ASC-US**)  
cannot exclude HSIL (**ASC-H**)
- ❖ Low-grade squamous intraepithelial lesion (**LSIL**)
- ❖ High-grade squamous intraepithelial lesion (**HSIL**)
- ❖ Squamous cell carcinoma

### **Glandular Cell Abnormalities**

- ❖ Atypical glandular cells (**AGC**) cervical, endometrial, NOS
- ❖ Atypical glandular cells, favor neoplastic
- ❖ Endocervical adenocarcinoma in situ (**AIS**)
- ❖ Adenocarcinoma

# Management of Cervical Cytologic Abnormalities...

## Considerations for Rational Plan of Conservative Management

- ❖ Intra- & inter-observer variation in diagnosis, especially at the lower end of spectrum
- ❖ Proportion of women with true premalignant disease and invasive cancer within each category
- ❖ Role of HPV DNA testing?
- ❖ Role of colposcopy?

# Reproducibility of Cytologic Interpretations

## Index Pap test classified as LSIL

- ❖ 68% LSIL
- ❖ 26% downgraded to ASCUS/ negative
- ❖ 6% upgraded to HSIL

## Index Pap test classified as HSIL

- ❖ 47% HSIL
- ❖ 27% downgraded to LSIL
- ❖ 23% downgraded to ASCUS
- ❖ 3% downgraded to negative

by quality control pathology group

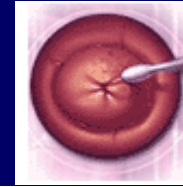
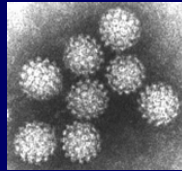
# Association of Cytologic Abnormalities with Biopsy confirmed findings

	ASC	ASC-H	LSIL	HSIL	AGC
CIN 2, 3	5 - 17%	20 - 40%	15 - 30%	70 - 75%	10 - 40%
Invasive Cancer	0.1- 0.2%		<0.1%	1- 2%	1- 9%

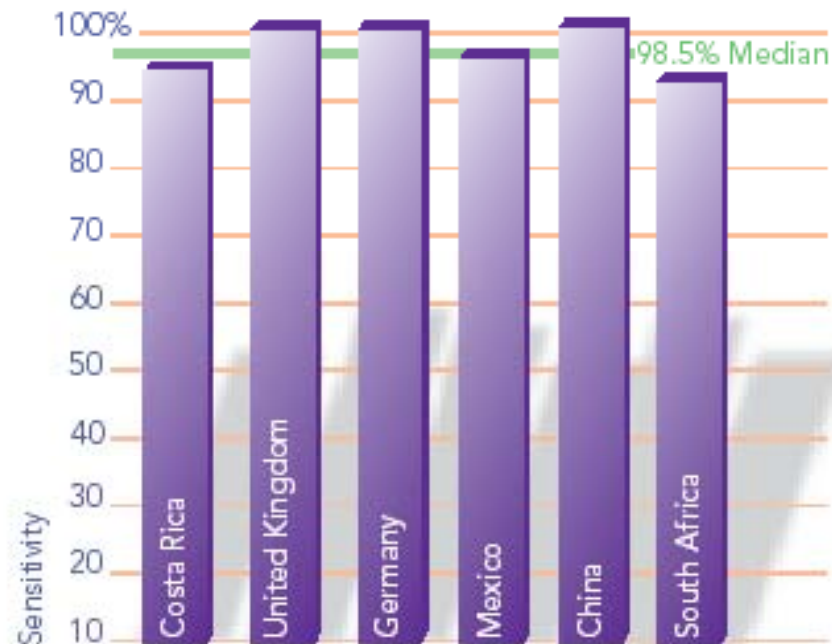
# Tools available for Assessment following Detection of Cytologic Abnormality

	Single Repeat Cytology	Serial Repeat Cytology	HPV Oncogenic types
Sensitivity	67-76%	2 smears: 91% 3 smears: 97%	96%
Referral for Colposcopy	-	59%	56%
NPV	-	96%	99%

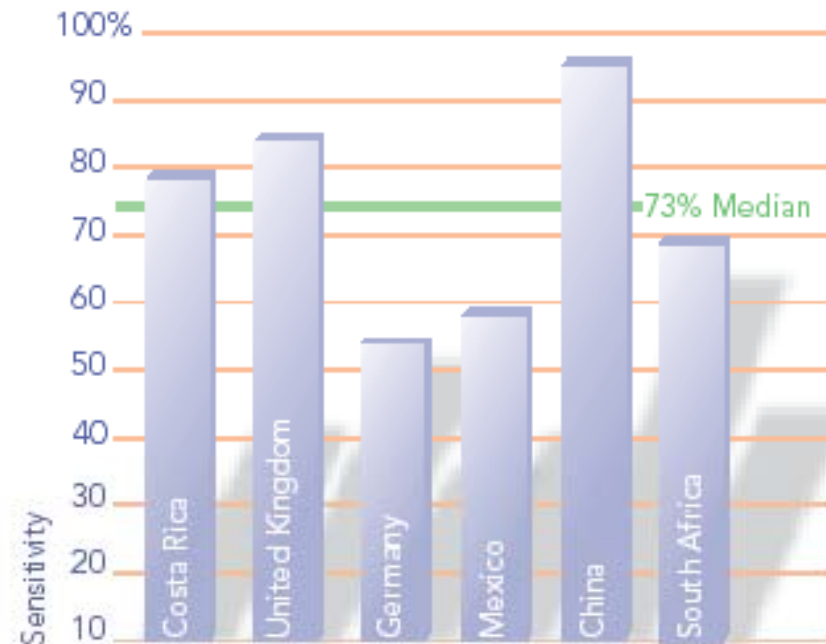
# Sensitivity for High-Grade Cervical Disease or Invasive Cancer



## The Digene HPV Test and Cytology vs. Cytology Alone



The Digene HPV Test and Cytology



Cytology Alone

# WHY Treat CIN?

- ❖ **Definite Risk of Progression**
- ❖ **Expertise of Colposcopist?**
- ❖ **Expertise of Pathologist ?**

# Philosophy of CIN Treatment

- **Local disease, Underlying stroma & lymphatics not involved**
- **Local removal / destruction of tissue (abnormal) is sufficient, provided it is assured that all tissue up to 5-7 mm depth is removed / destroyed**

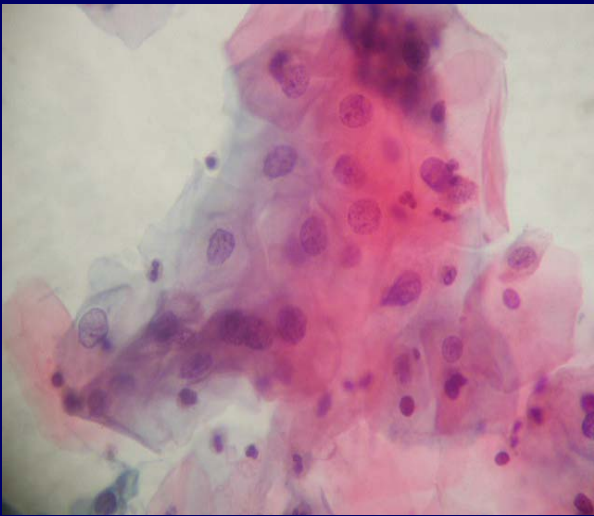
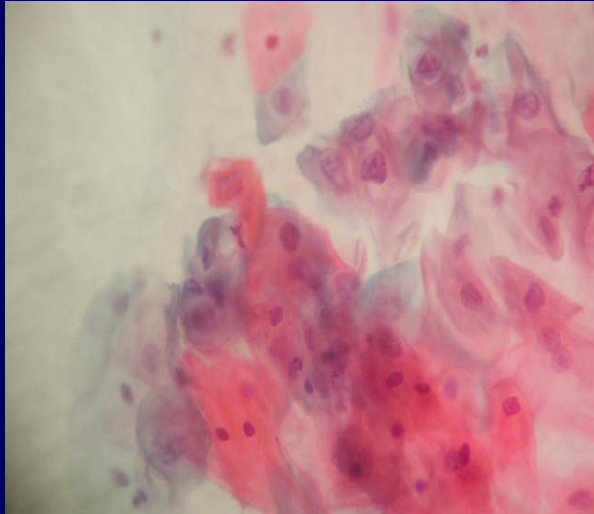
# Decision Regarding Treatment

- ❖ **Age of the patient**
- ❖ **Desire for fertility**
- ❖ **Size & Grade of lesion**
- ❖ **Co-existent pathology**
- ❖ **Facilities available**
- ❖ **Feasibility of follow-up**

# Management of ASC-US smear

## 3 Options

- ❖ Repeat Pap smear in 4-6 months
  - If  $\geq$  ASC-US... refer for colposcopy
  - 3 consecutive -ve smears... Routine screening
- ❖ Immediate Colposcopy
  - If -ve ...repeat cytology at 12 months
  - If biopsy proven lesion... manage accordingly
- ❖ High Risk HPV testing
  - + ... refer for colposcopy
  - - ... for CIN...Repeat cytology or/ HPV at 12 mths



# Management of ASC-H smear

- \* Colposcopy Recommended for All women with ASC-H

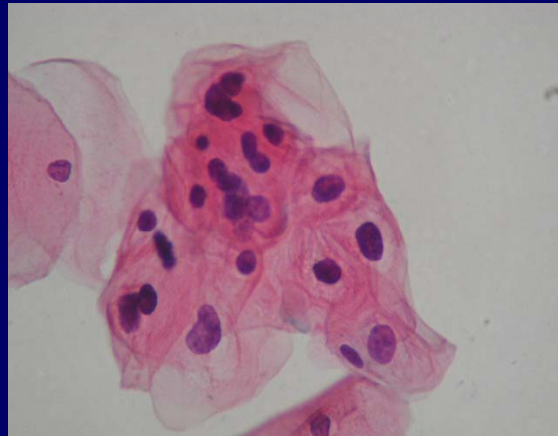
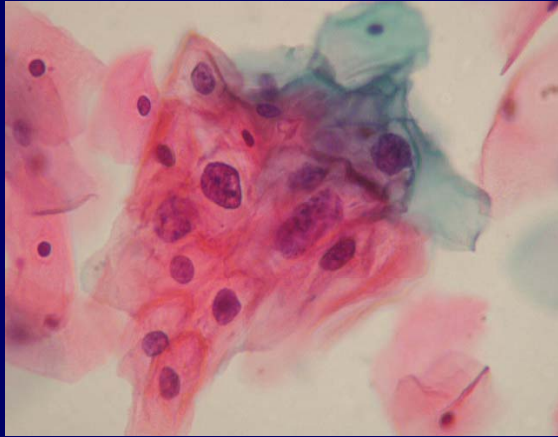


If no lesion... Review cytology, HPV, colposcopy

- \* If interpretation remains ASC-H, Repeat HPV DNA at 12-24 months
- \* Conization not indicated for cytology/ histology discrepancy with ASC-H diagnosis

**LEEP "See & Treat" should not be used for ASC without biopsy proven CIN because of potential for over-treatment**

# Management of LSIL smear



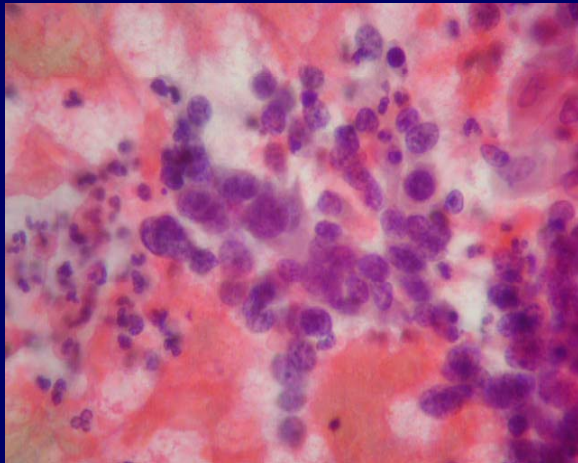
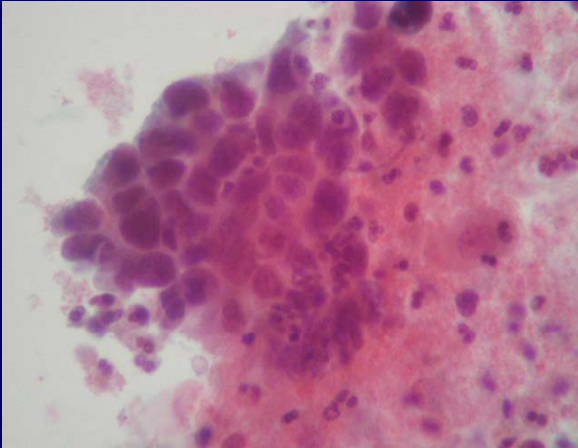
- ❖ **Colposcopy recommended for all women with LSIL**
- ❖ **If lesion in TZ & Colposcopy satisfactory → Directed biopsy**
- ❖ **If no lesion/ unsatisfactory → Review cyto-, HPV & ECC**
- ❖ **If biopsy fails to reveal CIN, follow up with Repeat cytology at 6 & 12 mo/ HPV DNA at 12 mo**

**Adolescents: Follow up with repeat cytology/ HPV testing is an acceptable option without initial colposcopy**

# Management of HSIL smear

- ❖ **Colposcopy with EC assessment is recommended for All women with HSIL**
  - Further management depends on colposcopy findings
  - \* **Satisfactory colposcopy with high grade lesion: Directed biopsy, LEEP or selective see & treat**
  - \* **Lower grade lesion or No lesion: Conization is preferred, except in pregnant & young reproductive age women**
  - \* **Unsatisfactory - Diagnostic conization preferred. HPV DNA Testing.**

**Ablative treatment may be unacceptable.**



# Management of AGC smear

- ❖ **Initial Colposcopy (Endometrial sampling in >35yrs) is recommended for All women with AGC**
- ❖ **Follow up with Rpt cytology or HPV acceptable**
- ❖ **Subsequent evaluation if invasive disease not identified**

**AGC favor Neoplasia-Diagnostic conization**

**AGC No neoplasia: Repeat cytology 4-6 mthly till 4 -ve. HPV DNA Testing or colposcopy**

# ACS Guidelines<sup>1</sup>

- ❖ “HPV DNA testing has greater sensitivity than cytology for detecting clinically relevant lesions” and the “high negative predictive value resulting from concomitant screening with cytology and HPV DNA testing could safely permit increasing screening intervals, thus lowering costs.”

# **WORLD HEALTH ORGANIZATION AGENCY RECOGNIZES ROLE OF HPV TESTING IN CERVICAL CANCER SCREENING (April,04)**

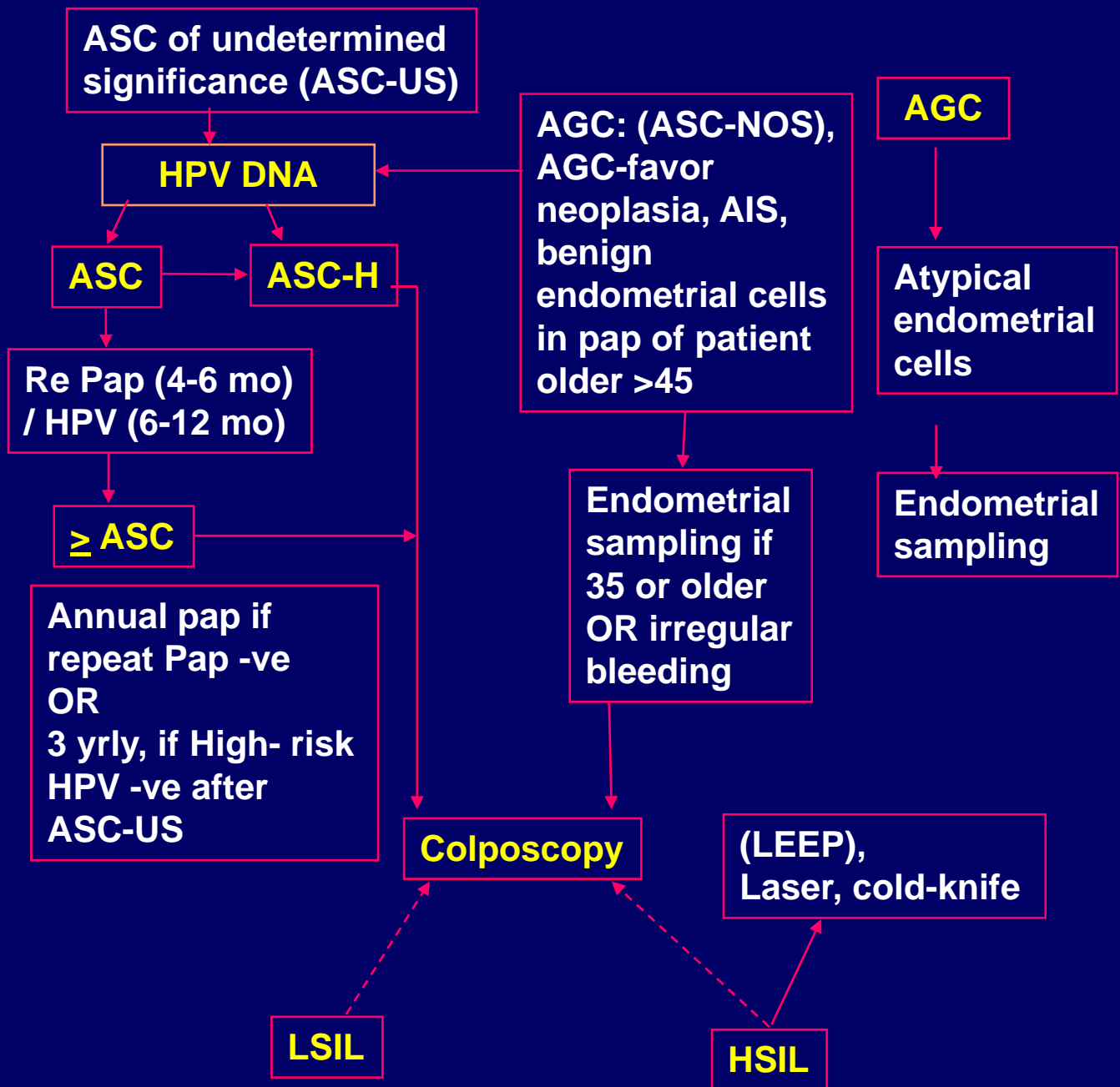
## **According to IARC:**

- HPV infections are a necessary cause of cervical cancer**
- Women can be considered virtually free of cervical cancer risk in the absence of persistent infection with high-risk types of HPV.**
- The use of “high-risk HPV types in screening & patient management is fully justified.**

# ACOG Recommendations

- ❖ **“The use of a combination of cervical cytology & HPV DNA screening is appropriate for women aged 30yrs & older. If this combination is used, women who receive -ve results on both tests should be rescreened no more frequently than every 3 or 5yrs.”<sup>1,2</sup>**

1. ACOG Press Release, July 31, 2003.
2. ACOG Practice Bulletin, Number 45, August 2003.  
Replaces Committee Opinion Number 152, March 1995.



➤ Post-menopausal women: ASC-US, LGSIL followed by hormonal treatment & repeat Pap.

➤ LSIL in adolescents may be followed by a Pap & HPV testing in 12 mths.

# Risk factors for failure after conization

## ❖ Resection margin with CIN

- Vaginal - endocervical
- Glandular lesions
- CIN grade

## ❖ Endocervical brush/curettage with CIN

## ❖ Treatment method

- LEEP
- Cold knife conization - laser cone
- Competence of resident

## ❖ Monocentric - multicentric CIN

# Guidelines for F/U after conization

## ❖ **United Kingdom**

- 6x Pap smears in 5yrs

## ❖ **Netherlands**

- Pap smear after 6, 12, 24 mo
- Colposcopy in case of abnormal Pap

## ❖ **Germany**

- Pap smear 3, 6, 9, 12, 18, 24 mo
- Colposcopy in case of abnormal Pap

# Optimal Method of Predicting Clearance?

## Pap smear

quite often (+), but  
only in 50% CIN

## Colposcopy

often inadequate  
difficult to interpret

## HPV DNA testing?

HPV eradication after tx  
Persistence required for CIN/SCC  
HR HPV present in post-tx CIN

# HPV Testing after CIN Treatment

- **Study Design**

- 104x CO2 laser conizations
- 67% CIN 2/3 (of which 84% HPV PCR +)

- **Results**

- No recurrence after 3 years
- 3% HPV (+), but different type

=>Laser cleared CIN and HPV

=>No residual disease

# HPV Testing after CIN Treatment

- **Study design:**

- 56x conization for CIN 3
- Follow up 12-73 mo

- **Results:**

- 9% (5/56) recurrent CIN
- 20% HPV (PCR) positive
- HPV sensitivity 100%; specificity = 55%

=>All HPV (-) ->no CIN recurrence

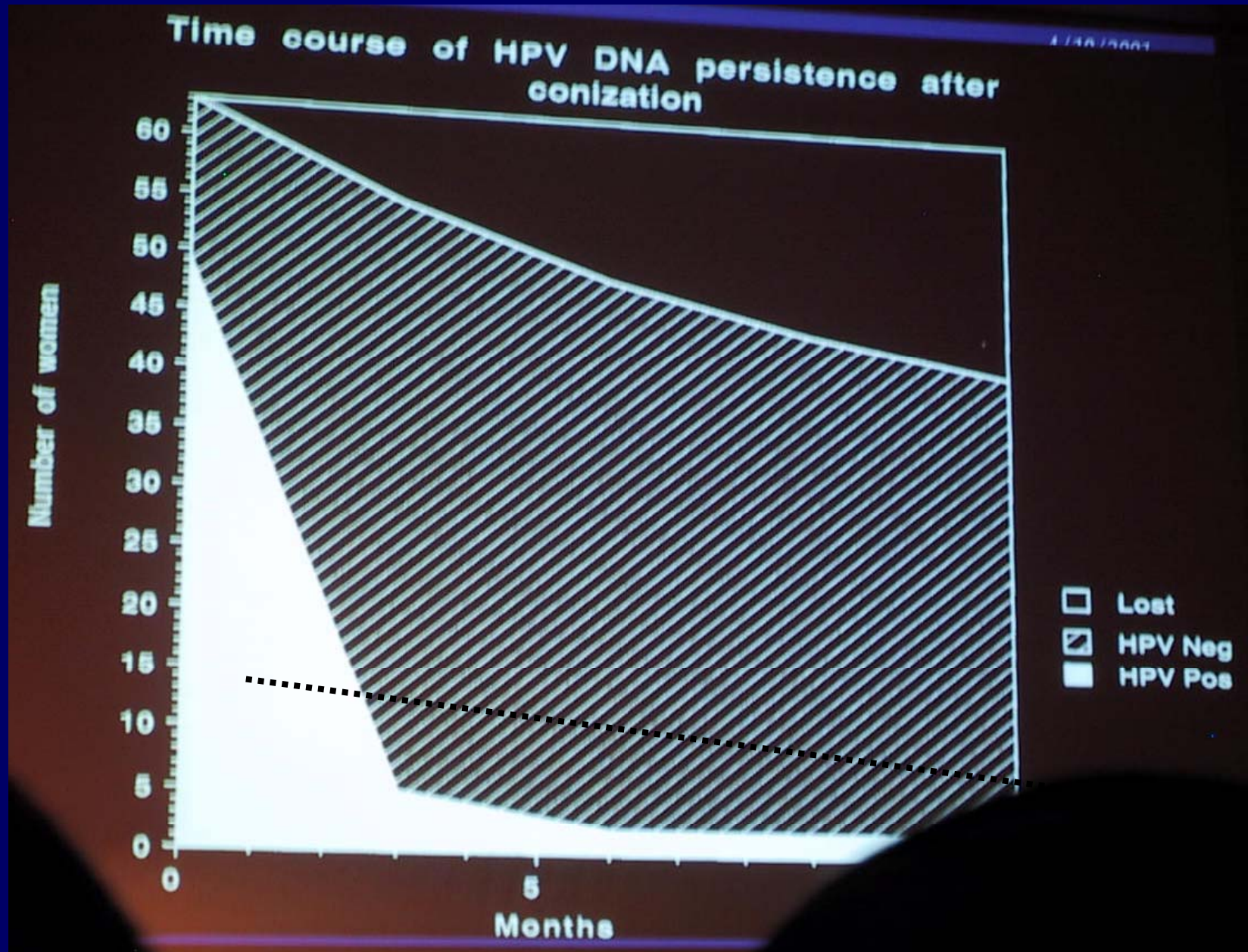
=>Persistent HPV infection = increased risk ( $p < 0.0001$ )

# HPV Testing after CIN Treatment

Post-tx CIN 2/3	Sensitivity		Specificity	
	HPV <sup>#</sup>	Pap	HPV	Pap
3 mo <sup>**</sup>	93%	58%	86%	91%
6 mo <sup>*</sup>	90%	62%	92%	91%
12 mo	90%	72%	96%	95%
24 mo	93%	93%	99%	96%
1 SCC and 1 CIN 3 overlooked by Pap for >24 mo but HPV 16 (+)				

All 21x HR-HPV (+) w/o post-tx CIN 2/3 cleared HPV in 4-18 mo  
 # 90% same HPV type

# HPV Clearance with Time after Conization: ~10% Residual Disease



# Colposcopy in Management of Cn Cx: Issues

- **HIGH LEVEL OF CONFIDENCE:** Weighted mean sensitivity to detect normal to abnormal cervical tissues- 0.98 & weighted mean specificity 0.49 (by the experts, also normal histology was not available in majority of cases as reported in literature)

## **BUT**

- It is inconvenient and uncomfortable
- Colposcopy referral may raise false concerns of cervical disease among patients
- Expensive
- Potential for over Dx & over Rx

# Limiting Factors For Satisfactory Colposcopy

- ❖ Inappropriate triage & evaluation i.e deviation from diagnostic protocol
- ❖ Colposcopic errors
  - Inadequate expertise
  - Over interpretation of minor lesions, size of the lesion (Re: ALTS)
  - Failure to record colposcopic findings
  - Pregnancy
  - Incomplete visualization of TZ
- ❖ Failure to inspect associated lesion over vulva & vagina

# Take Home Message

- ❖ **10% disease after CIN treatment**
- ❖ **Increased incidence of cervical cancer after CIN 3 conization!**
- ❖ **HPV more sensitive than Pap smear**
- ❖ **HPV specificity appears similar**
- ❖ **Optimal time lag needs validation by other studies**
  - **Cost-benefit analysis**
  - **Larger studies necessary**



**THANK YOU**