Histopathologic evaluation of cervical intraepithelial neoplasia (CIN) in tissue biopsy samples: Dilemmas and solutions

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Cervical carcinoma is a global burden that occurs more frequently in developing countries of Africa, Asia and Latin America compared to the industrialized world of Europe and North America. The incidence of cervical carcinoma correlates with the socioeconomic status of a population and has been strongly influenced by the implementation of cytological screening that allow the early detection of cervical precursor lesions. Organized screening programs such as in Scandinavian countries, the Netherlands and U.K. are associated with a lower incidence and mortality of cervical cancer compared to opportunistic screening like in Austria. The etiology and pathogenesis of cervical cancer is strongly linked to HPV. Almost all types of cervical cancers have HPV integrated within their genome. For the most frequent types of cervical carcinoma, squamous cell carcinoma and adenocarcinoma distinctive precancers are known. Squamous cell carcinoma is considered to develop from a precancer designated cervical intraepithelial neoplasia (CIN). CIN is graded into 3 groups, CIN 1−3 according to the degree of proliferation of atypical basal cells and the presence of mitotic figures. For grading of CIN the squamous epithelium is divided into 3 thirds. The atypical basal and parabasal cells involve the basal third in CIN 1, the basal and the middle third in CIN 2 and more than two thirds in CIN 3. In particular, CIN 1 and 2 are not well defined since the presence of mitosis as well as koilocytotic changes are considered further diagnostic criteria. For the diagnosis of CIN 3 the presence of mitoses in the superficial third of the epithelium is considered helpful. In contrast to CIN 1 and also CIN 2, CIN 3 lacks a significant amount of koilocytes. The Bethesda system, which was originally established for cervical cytology uses only two categories of HPV associated lesions, low and high grade squamous intraepithelial lesions (LSIL and HSIL) that are considered biologically distinctive. LSIL is characterized by extensive koilocytosis and a proliferation of immature, undifferentiated cells within the lower third of the epithelium. Abnormal mitosis are rare. Any anogenital HPV type may occur in LSIL and the lesions are usually diploid or polyploid. In contrast, HSIL shows a proliferation of

undifferentiated, immature cells that involves at least the middle third of the epithelium and koilocytes are scant. HSIL are usually aneuploid and associated with high risk HPV such as HPV 16. Compared to the WHO system LSIL correlates with CIN1, whereas CIN2 and 3 are summarized under HSIL.

There are several problems with CIN lesions:

1. It is not clear how frequently CIN of various grade progress and show regression, respectively;
2. In particular, CIN 2 shows poor agreement among observers, even among experts;
3. There are further problems with other lesions, particularly the distinction between CIN 1 and reactive and CIN 3 and metaplastic, respectively;
4. The role of adjunct methods, in particular biomarkers for diagnostic purposes needs to clearly defined.

The tendency of progression and regression of the various lesions varies between different studies. It ranges between 10 and 70% for CIN 1 and CIN 3, respectively. However, larger studies such as the Toronto Long term Follow up study for abnormal cytology revealed a more than 4-fold increased progression rate for CIN 3 compared to CIN 1. However, it is likely that even CIN 2 show a greater progression rate than expected. 40 % of underdiagnosed CIN 2 seem to regress but progression seems to depend on the type of HPV, in particular HPV 16.

The reproducibility of CIN 3 is significantly better compared to CIN 2 as shown by various groups. CIN 3 seems to be more frequently associated with oncogenic HPV and abnormal cytology compared to CIN 2. CIN 2 seems to be both undercalled as CIN 1 and overcalled as CIN 3. Therefore, suggestions have been made to request a second opinion for histological diagnosis of CIN 2. CIN 2 is frequently associated with HPV 16 but it is unclear which CIN 2 show progression to CIN 3 and invasive carcinoma, respectively. Recently, p16 immunhistochemistry was suggested as diagnostic adjunct for histological diagnosis of CIN2.

The implementation of p16 immunohistochemistry in daily routine seems to be of great diagnostic value in the grading of cervical lesions on biopsies. There is evidence, that the combination of HE and p16 immunhistochemistry is as good as an expert second opinion. A

diffuse strong staining is typical for CIN 3 and most CIN 2, the latter possibly also confined to the basal two thirds of the epithelium. In contrast, CIN 1 shows a focal, patchy staining. Reactive epithelium is often completely negative. P16 is also diffusely positive in adenocarcinoma in situ (AIS) and most invasive adenocarcinomas. Ki-67 may be used in combination with p16 but obviously does not add any advantage. L1 protein immunohistochemistry has not been widely accepted for routine diagnosis, so far.

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Histopathologic Evaluation of Cervical Intraepithelial Neoplasia (CIN) in Tissue Biopsy Samples: Dilemmas and Solutions

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Global Incidence of cervical carcinoma (2000)

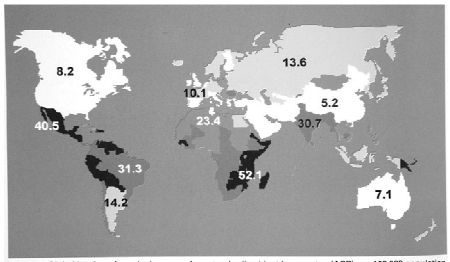
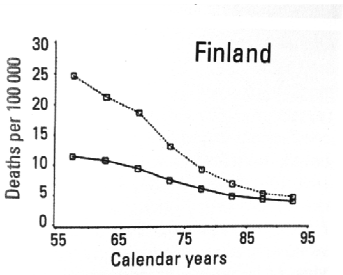


Fig. 5.01 Global burden of cervical cancer. Age-standardized incidence rates (ASR) per 100,000 population and year. From Globocan 2000 (846).

Epidemiology of cervical carcinoma

- HPV
- Socio-economic status
- Dramatic decrease of the incidence by cervical cytology screening

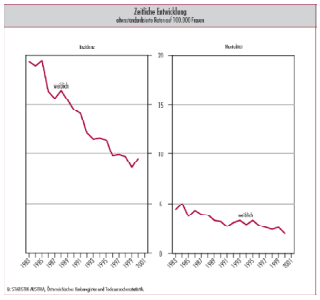
Incidence and Mortality of Cervical Carcinoma in Finland



WHO: Tumours of the Female Genital Tract, 2003

Incidence and Mortality of Cervical Carcinoma in Austria 1983-2001

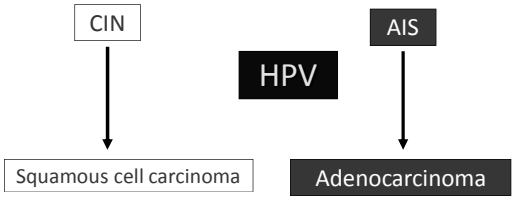
2007: <6



2007: 2

Statistik Austria, 2004

Cervical carcinoma and precursor



Classification of Squamous Precursor Lesions

Traditional	WHO	Bethesda
Mild dysplasia	CIN1	LSIL
Moderate dysplasia	CIN2	HSIL
Severe dysplasia	CIN3	
Carcinoma in situ		

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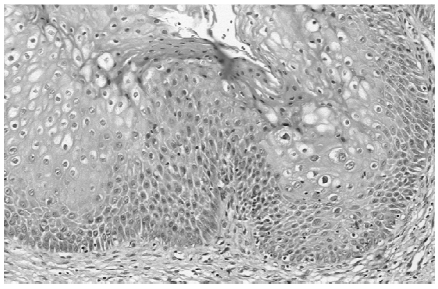
Cervical Intraepithelial Neoplasia 1 (CIN 1)



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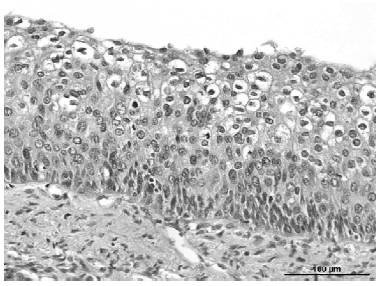
Cervical Intraepithelial Neoplasia 2 (CIN 2)



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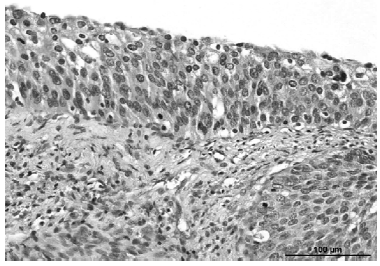
Cervical Intraepithelial Neoplasia 3 (CIN 3)



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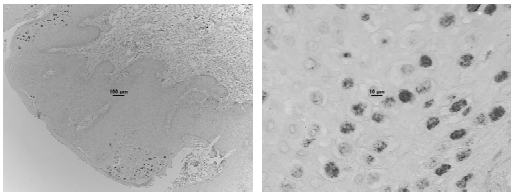
HPV DNA demonstrated by in situ Hybridisation



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First exposure to HPV16 and development of high grade CIN

Woodman et al., Lancet 2001

Time since first exposure (months)	Relative hazards ratio (95% CI)*
Unexposed	1·00
≤6	5·98 (1·33–26·85)
6–12	18·02 (5·50–59·03)
12–18	14·22 (3·76–53·86)
>18	2·60 (0·75–8·99)

HPV=human papillomavirus. CIN=cervical intraepithelial neoplasia. *Controlling for any other HPV exposure.

Table 4: **Risk of high-grade CIN in relation to time since first exposure to HPV 16**

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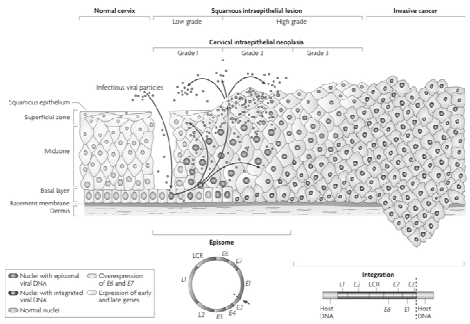
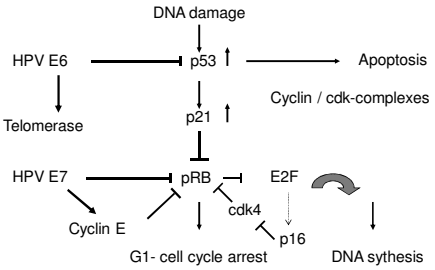


Lax, ZORA Meeting 2012, Brdo Woodman et al., Nature Rev 2007

Molecular mechanisms of HPV on the host cell



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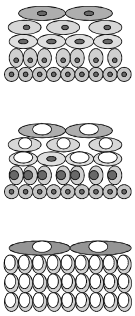
Cell Transformation in Squamous Intraepithelial Lesions



Terminally differentiated cells

Committed cells
Basal and stem cells

LSIL / CIN 1

Transformed terminally differentiated cells

Committed cells
Basal and stem cells

HSIL / CIN 2&3

Lack of terminal differentiation

Transformed basal/stem and committed cells

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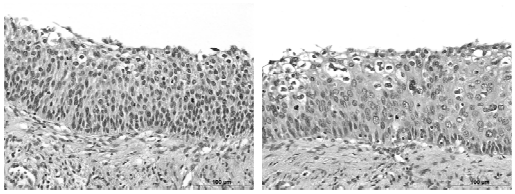
2 lesions from the same patient

CIN3 / HSIL **CIN2 / HSIL**



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Distinguishing Features of L/HSIL

Wright et al., in: Blaustein, 6th ed., 2011

Feature	LSIL	HSIL
HPV type	Any anogenital	High risk
Koilocytosis	Frequent	Rare
Ploidy	Diploid or polyploid	Most aneuploid
Abnormal mitosis	Absent	Frequent
Location undifferentiated cells/mitosis	Lower third	Upper 2 thirds

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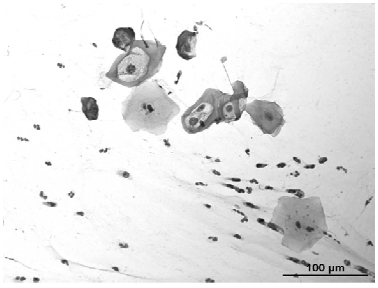
LSIL



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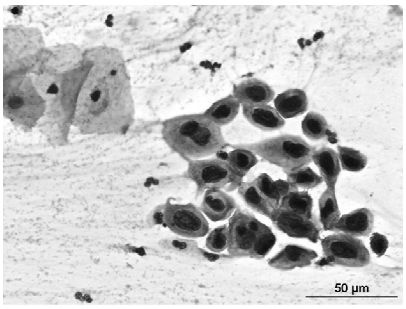
HSIL



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Problems and Questions

- Diagnostic uncertainty due to poor reproducibility, in particular of CIN 2
- Further diagnostic weaknesses:
  - Reactive versus CIN1
  - Reactive/metaplastic versus CIN3
- Uncertain potential of progression of the various lesions
- Biomarkers for improved diagnostic accuracy

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Natural History of Various CIN

	% Regression	% Persistence	% Progression
CIN1	57	32	11
CIN2	43	35	22
CIN3	32	56	12

Mitchell et al., JNCI 1996

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Toronto Long Term Follow up of Abnormal Cytology

Holowaty et al., JNCI 1999

Degree Dysplasia	% Progression 2/10 y	% Regression 2/10 y
Mild	0.6 / 12	44 / 88
Moderate	1.5 / 17	33 / 83
Severe	2.8 /21	

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Interobserver Agreement for CIN

- Varies among different studies
- Substantial disagreement due to problems with CIN1 and CIN2
- Improved results by using weighted k-statistics

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The Natural History of CIN2

Castle et al., Gynecol Oncol 2009

- 40% of undiagnosed CIN2 seem to regress within 2 years
- CIN2 containing HPV16 seems to progress more likely compared to CIN2 with other HPV types

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Reproducibility of CIN between experts

Carreon et al., Int J Gynecol Pathol, 2007

- Population-based study in Costa Rica
- Comparison of local pathologists and 2 experts
- Diagnosis correlated with HPV and cytology
- CIN3: 81/84%, CIN2: 13/31% agreement
- Oncogenic HPV: 94% (CIN3) and 72% (CIN2)
- Abnormal cyto: 81% (CIN3) and 61% (CIN2)
- CIN3 is better reproducible and can better validated by HPV test and cytology than CIN2

Reproducibility of CIN between experts

Carreon et al., Int J Gynecol Pathol, 2007

TABLE 3. *Concordance of diagnoses between 2 secondary quality assurance pathologists*

NCI reviewer 1 diagnoses	NCI reviewer 2 diagnoses				
	Negative, n (%)	CIN1, n (%)	CIN2, n (%)	CIN3, n (%)	Total, n (%)
Negative	45 (91.8)	4 (8.2)	0 (0)	0 (0)	49 (55.1)
CIN1	8 (32.0)	12 (48.0)	5 (20.0)	0 (0.0)	25 (28.1)
CIN2	1 (25.0)	2 (50.0)	0 (0)	1 (25.0)	4 (4.5)
CIN3	0 (0)	0 (0)	3 (27.3)	8 (72.7)	11 (12.4)
Total, n (%)	54 (60.7)	18 (20.2)	8 (9.0)	9 (10.1)	89 (100)

Weighted $\kappa = 0.71$ (95% CI, 0.61–0.82).

Interobserver Agreement for CIN

Cai et al., AJSP 2007

- 4 experts; QC slide panel (n=185)
- Both inter- and intraobserver variability good among experts if weighted k-values used (0.75-0.86 and 0.74-0.94, respectively)
- Best for non-CIN and CIN3
- Worst for CIN2
- Disagreement more frequent between neighbouring categories

TABLE 1. Characteristics of the Quality Control Slides

Difficulty of Slides	"Gold Standard" Diagnosis				
	Negative	CIN 1	CIN 2	CIN 3	Total
Gold standard reached unanimously	60 (84.5%)	6 (31.6%)	7 (19.4%)	28 (47.5%)	101 (54.6%)
Gold standard reached through consensus meeting	11 (15.5%)	13 (68.4%)	29 (80.6%)	31 (52.5%)	84 (45.4%)

Interobserver Reproducibility of CIN2

Cai et al., AJSP 2007

TABLE 3. Interobserver Agreement by Category of Diagnosis

	No. Paired Observations				Category-specific κ (Range)
	Non-CIN	CIN 1	CIN 2	CIN 3	
Non-CIN	**760**	59	23	18	0.81 (0.79-0.84)
CIN 1		**161**	38	1	0.57 (0.52-0.63)
CIN 2			**157**	113	0.38 (0.33-0.44)
CIN 3				**643**	0.74 (0.71-0.77)
			Overall weighted κ		0.80 (0.78-0.82)

The diagnostic problem of CIN2

- Mixture of CIN1 (1/3) and undercalled CIN3 (2/3) (Castle et al. 2007)
- Overcall of CIN1 more likely?! (Galgano et al., 2008)
- Unclear, which CIN2 progress
- High prevalence of HPV16 (43%)
- 2nd opinion for CIN2 recommended to increase diagnostic accuracy
- p16 immunohistochemistry as aid? (Dijkstra et al., 2010)

HPV16 Genotyping a Benchmark for Cervical Biopsy Interpretation ?

Galgano et al. AJCP, 2008

- ALTS population (ASCUS+LSIL); n=5060
- 10 centers+expert panel
- Hybrid Capture 2 and HPV genotyping
- % of HPV16 positivity correlated with severity of lesion
- But significant discrepancy between centers
- Agreement between centers and experts weak regarding CIN2</>

HPV16 Genotyping a Benchmark for Cervical Biopsy Interpretation ?

Galgano et al. AJCP, 2008

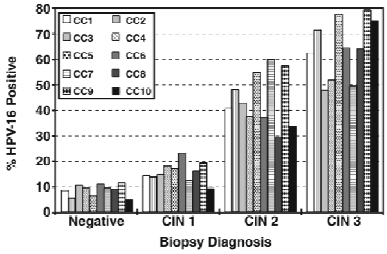
Table III
Comparison of Paired Biopsy Diagnoses by Two Pathology Groups in Relation to the Percentage of hc2+ and the Percentage of HPV-16+ as Detected by the Line Blot Assay*

	QC Pathology						
Clinical Cancer Pathology	Negative	Atypical	CIN 1	CIN 2	CIN 3+	Total	P
Negative							
No. (%) of cases	2,302 (51)	9 (2)	24 (2)	10 (1)	4 (0)	2,351 (42)	
hc2+ (%)	43	63	61	80	80	44	
HPV-16+ (%)	6	0	9	14	25	6	
Atypical							
No. (%) of cases	469 (9)	35 (1)	45 (1)	11 (1)	7 (0)	569 (10)	
hc2+ (%)	67	69	67	73	100	68	
HPV-16+ (%)	10	12	8	0	29	11	
CIN							
No. (%) of cases	568 (12)	22 (1)	786 (16)	115 (2)	23 (0)	1,526 (31)	
hc2+ (%)	82	95	87	93	91	76	.02
HPV-16+ (%)	14	9	7	21	43	10	.003
CIN 2							
No. (%) of cases	25 (1)	1 (0)	110 (2)	241 (5)	147 (3)	546 (11)	
hc2+ (%)	61	67	95	92	96	91	.0
HPV-16+ (%)	15	13	31	44	59	42	< .0001
CIN 3+							
No. (%) of cases	7 (0)	4 (0)	6 (0)	62 (1)	210 (4)	289 (6)	
hc2+ (%)	71	80	100	95	97	96	.9
HPV-16+ (%)	33	25	60	53	67	62	.27
Total							
No. (%) of cases	3,394 (61)	83 (2)	983 (20)	444 (9)	391 (8)	4,915	
hc2+ (%)	45	67	86	94	96		
HPV-16+ (%)	10	10	9	37	61		
P							
hc2+ (%)			.001	.2	.4		
HPA-16+ (%)			< .0001	< .0001	.02		

CIN, cervical intraepithelial neoplasia; hc2, Hybrid Capture 2; HPV, human papillomavirus; QC, quality control.
* P values are for trend tests among women who had at least CIN 1 as diagnosed by both pathology groups.

HPV16 Genotyping a Benchmark for Cervical Biopsy Interpretation ?

Galgano et al. AJCP, 2008



P16 immunostaining as an alternative to histology review for reliable grading of CIN

Dijkstra et al., J Clin Pathol 2010

- Combined use of HE and p16 ImHC significantly improves accuracy of interpreting and grading cervical lesions on biopsies
- Accuracy of CIN grading of a single pathologist with p16 adjunct comparable to expert panel
- P16 staining of cervical lesions should be implemented in daily routine

P16 immunostaining as an alternative to histology review for reliable grading of CIN

Dijkstra et al., J Clin Pathol 2010

Table 1 Kappa values for agreement between pairs of pathologists before and after additional interpretation of $p16^{INK4a}$ stained sections

All histological categories	H&E-based diagnosis	95% CI	p16-supported diagnosis	95% CI
Kappa values (weighted)				
PA1 versus PA 2	0.44	0.19 to 0.64	0.82	0.52 to 0.92
PA1 versus PA 3	0.66	0.47 to 0.79	0.80	0.67 to 0.88
PA 2 versus PA 3	0.53	0.29 to 0.70	0.79	0.54 to 0.91
Group (mean) kappa	0.54	0.38 to 0.69	0.80	0.66 to 0.89
Kappa values (unweighted)				
Two categories (=CIN1−CIN2/3)				
PA 1 versus PA 2	0.32	0.04 to 0.55	0.80	0.66 to 0.88
PA 1 versus PA 3	0.64	0.44 to 0.78	0.67	0.48 to 0.80
PA 2 versus PA 3	0.37	0.11 to 0.58	0.80	0.67 to 0.88
Group (mean) kappa	0.44	0.27 to 0.60	0.76	0.64 to 0.84

CIN, cervical intraepithelial neoplasia; PA, pathologist.

P16 immunostaining as an alternative to histology review for reliable grading of CIN

Dijkstra et al., J Clin Pathol 2010

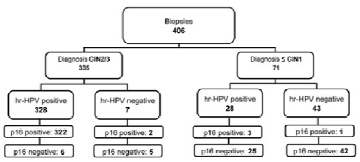


Figure 2 High-risk human papillomavirus (hrHPV) status and $p16^{INK4a}$ expression in relation to the consensus diagnosis. Results of both hrHPV DNA testing and immunohistochemical analysis of the $p16^{INK4a}$ protein expression (dichotomised into 'positive' (scores 3/4) and 'negative' (scores 0/1/2)), on 406 biopsy samples in relation to the histology grade according to the consensus diagnosis. CIN, cervical intraepithelial neoplasia.

"Surrogate markers" for CIN diagnosis

- P16
- Ki-67
- HPV
- L1

p16INK4

- Overexpression in CIN, AIS and most carcinomas;
- Association with HPV: RB Inactivation ?!
- Good correlation with SIL/CIN
- Detection of dysplastic cells in Pap smears (Klaes et al. Int J Cancer 2001)

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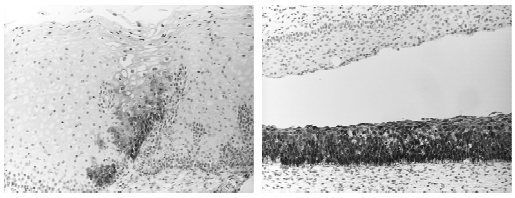
P16 in low and high grade CIN



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p16 and CIN

Klaes et al., AJSP 2002, Branca et al., IJGP 2004, Tringler et al. Hum Pathol 2004

- Diffuse positive p16 immunoreactivity only in invasive carcinomas, CIN2/3 and CIN1 associated with high risk HPV
- Part of CIN 1-3 negative for p16
- No predictive value for high risk HPV clearance after conisation, no prognostic value for carcinomas
- Positivity also in reactive and metaplastic epithelium

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Ki-67 (Mib 1)

- Expression during the cell cycle
- In normal epithelium expression only in suprabasal and a few basal cells (hormone dependence)
- HPV infection leads to activation of the cell cycle
- LSIL: positive cells in the superficial third of the epithelium (not found in reactive changes)
- HSIL: multple positive cells throughout the epithelium
- Assist in the distinction of SIL from reactive changes !

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Pap III / ASCUS-H

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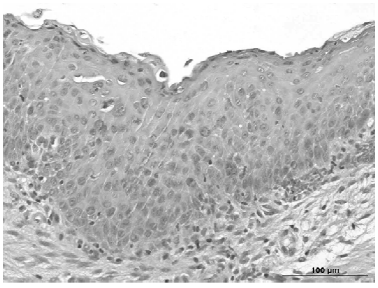
Atypical Squamous Epithelium



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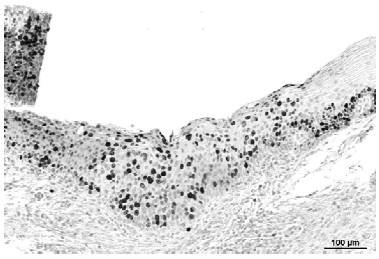
Ki-67



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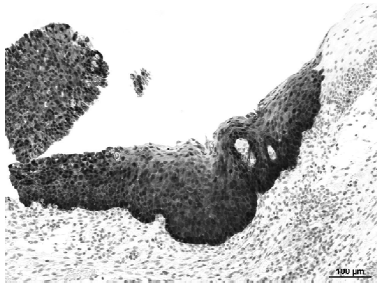
p16



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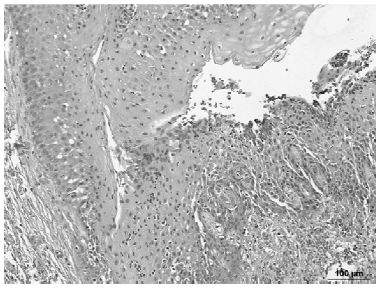
Reactive or CIN



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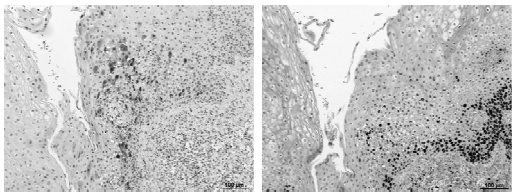
Reactive Changes

p16 **Ki-67**



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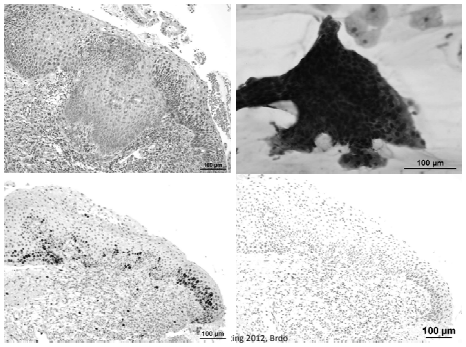
Reactive versus neoplastic



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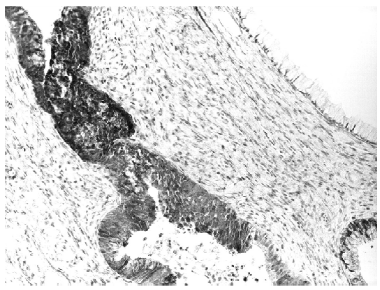
P16 is overexpressed in AIS



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Cervical Lesions and P16

Galgano et al., AJSP 2010

- P16 more sensitive than HE histology
- For the distinction between CIN and reactive/metaplastic changes reliable
- Ki-67 seems to provide no additional information ?
- P16 seems to be particularly helpful for CIN2

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Immunohistochemistry HPV L1

Negri, AJSP 2008, Galgano, AJSP 2010; Hoshikawa, Path Res Pract 2010

- Not widely in use; in combination with p16?
- Specific proof of HPV L1 capsid protein
- Indicates productive phase
- Prognostic value for CIN1?
  - L1 positive: 21-27% progression
  - L1 negative: 80-97% progression
- No distinction between CIN1 and reactive

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Combination of L1 and p16

Negri et al. AJSP, 2008

TABLE 3. Combined Expression of p16 and L1 in CIN1

	p16 + L1 −	p16 + L1 +	p16 − L1 +	p16 − L1 −
Group A (n = 38)	26 (68.42%)	12 (31.58%)	—	—
Group B (n = 28)	1 (3.57%)	7 (25%)	4 (14.29%)	16 (57.14%)

Group A: cases with coexistent CIN1 and CIN3. Group B: CIN1 with spontaneous regression. CIN indicates cervical intraepithelial neoplasia.

- Many L1-/p16- lesions seem to be reactive
- Assessment of the progression potential of CIN1?

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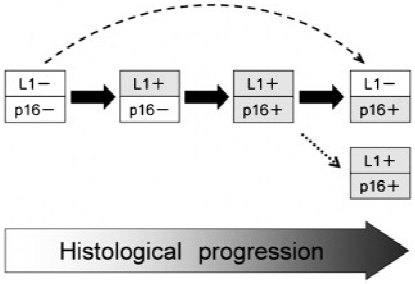
L1, p16 and Progression

Hoshikawa et al., Path Res Pract 2010



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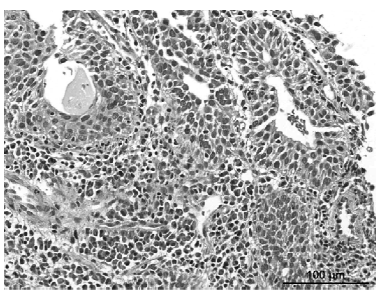
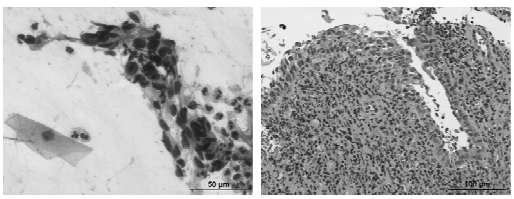
Caveat of high grade CIN

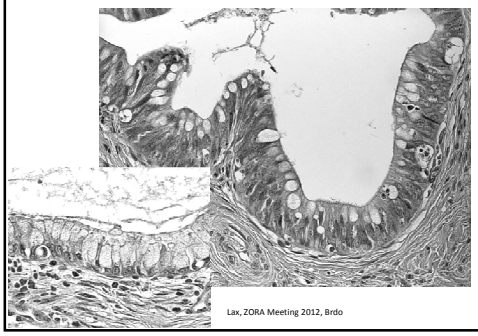


High grade CIN in crypts/glands may resemble atypical glandular epithelium

CIN3 as glandular mimic



Adenocarcinoma in situ (AIS)



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Adenocarcinoma in situ (AIS/ACIS)

- Normal glandular or surface epithelium replaced by neoplastic epithelium
- No invasion
- Concomitant CIN in ca. 50%
- Atypical Pap Smear only in 50%
- Dysplasia not used (poor reproducibility)
- CGIN: British terminology

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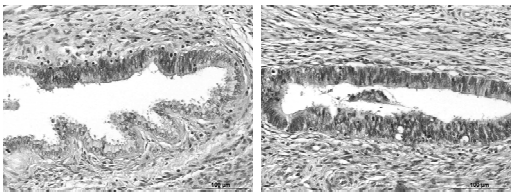
2 lesions of the same patient

Dysplasia? **AIS**



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Take Home Message

- Inter-/intraobserver agreement for CIN in particular valuable for negative and CIN3
- CIN2 is a problematic lesion
- 2nd opinion or p16 adjunct suggested
- P16 most important surrogat marker for HPV
- Add of Ki-67 not necessary but may be helpful
- HPV L1 Protein immunohistochemistry not widely used (informs about CIN1 progression)

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