



## Comparative study of p16ink4a expression in cytology and biopsy samples of cervical lesions

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### Introduction

There is a well known association between human papilloma virus (HPV), and cervical pre-neoplasia and neoplasia. Several studies indicate that certain virus types can cause genetical alterations via E6 and E7 oncogenes, leading to an over expression of p16 protein. This can be demonstrated immunochemically using the monoclonal antibody p16<sup>INK4A</sup>. In this work we aimed to demonstrate the p16 reaction in all cytological cervical smears (in the year 2004) which were matched with a cervical biopsy and compare with the p16 reaction in the histological slides.

### Material and Methods

89 cases from the Gynecology Clinic met the criteria set above. 7 cases were excluded from the study because the biopsy did not include representative material from the cervical epithelium. Women ages varied from 19 to 75 years (mean 36.3). Grading of cytological lesions was achieved using the Bethesda system: HSIL, LSIL and ASCUS. Cytology slides were stained with Papanicolaou method, mostly using thin layer smearing (ThinPrep) from liquid based specimens. If no square smear was available, the stained slide was decolorized and the p16 reaction applied, using the Dako kit p16<sup>INK4A</sup> (cytology). Histology slides from paraffin blocks, were stained with H&E and graded according to the criteria of CIN I, II, III and negative CIN. The histological kit was applied to the histological preparations, for the p16 reaction.

A positive result for p16 in cytology smears was considered when 10 or more cells were stained (nucleus and cytoplasm) and for histology 5% or more of the cells marked in the lesion.

In addition, 34 cases had separate samples submitted by the gynaecologist for hybrid capture Hc2 HPV-HR.



Fig 1 A- HSIL, PAP stain, 400X. B- HSIL positive for p16, 400X.  
C- CIN II, H&E stain, 200X. D- CIN II positive for p16, 200X.



Fig 2 A- LSIL, PAP stain, 400X. B- LSIL positive for p16, 400X.  
C- CIN I, H&E stain, 200X. D- CIN I positive for p16, 200X.

### Results

The 61 cases that we used in our study (those that had biopsy with representative cervical epithelium), were distributed as follows, using The Bethesda System (TBS) of classification: HSIL-30, LSIL-26, ASCUS-5.

The agreement between the cytology diagnosis (TBS) and the histological grading (CIN) is presented in Table I.

In Table II, p16 reactions for cytological and histological samples are compared. HSIL and CIN I/CIN II were positive in 97% and 93% respectively. In LSIL and CIN I/HPV, there were 62% and 50% positive cases. Only 5 cases of ASCUS were available and the positive results were 60% and 20%.

Table III summarizes the results in the 34 cases which were submitted to Hybrid capture 2 - HPV-HR.

Table I - Total cases studied for p16 reaction

Cytology grade (TBS)	Matching CIN grade	Non matching CIN grade
HSIL (30)	27 (90%)	3 (10%)
LSIL (26)	15 (58%)	11 (42%)
ASCUS (5)	2 (40%)	3 (60%)
Total (61)	44 (72%)	17 (28%)

Table II - Comparison of p16 reactions matched Cytology / Histology paired samples.

	Cytology (TBS)		Histology (CIN)	
	p16+	p16-	p16+	p16-
HSIL/CIN II-III	29 (97%)	1 (3%)	28 (93%)	2 (7%)
LSIL/CIN I-HPV	16 (62%)	10 (38%)	13 (50%)	13 (50%)
ASCUS/CIN negative	3 (60%)	2 (40%)	1 (20%)	4 (80%)
Total nr. of cases	48 (79%)	13 (21%)	42 (69%)	19 (31%)

### Conclusions

- There was 90% agreement, in the high grade group, between the cytology grading and the matched CIN lesions in histology.
- In the lower grade and ASCUS groups, the agreement with histology was 58% and 40% respectively.
- There was high level of agreement and positivity for p16 reaction in the high grade groups of cytology and histology.
- In the lower grade group and ASCUS there were higher level of positivity for p16 reactions in cytology, although the small number available for the ASCUS group precludes significant conclusions.
- With the hybrid capture (Hc2 HPV-HR), there was similarity in the results when compared with p16 reactions. However, when the figures are broken down into the different grading groups, it appears that the p16 reaction is more sensitive in the HSIL group and less sensitive in the LSIL group.

Table III - Hybrid capture 2 (Hc2 HPV-HR) in cytology samples.

	Hc2 +		Hc2 -	
	n	%	n	%
HSIL	11 (65%)	9 (35%)		
LSIL	11 (73%)	4 (27%)		
ASCUS	2 (100%)	0 (0%)		
Total nr. of cases	24 (71%)	10 (29%)		

**References**  
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