

HPV detection as follow-up of low-grade lesions in the Swedish gynaecological screening program. Comparison between Hybrid Capture II and the RNA-based PreTect HPV-Proofer Assay.

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Introduction

In Sweden approximately 40 000 cytology cases pr. year show aberrations which needs follow-up. Most cases regress spontaneously but some progress if not treated. There is also a problem of low sensitivity for cytology in the follow-up procedure. In detection of pre-cancerous lesions, both specificity and sensitivity has been found to improve drastically when HPV testing is performed after detection of cytological ASCUS or CIN I.

Objective

The main objective was to evaluate the respective roles of HPV RNA and DNA tests in relation to cytology and histology in the Swedish screening program. Another important objective was to estimate the risk of missing CIN II+ in women with CIN I or ASCUS but negative with either HPV RNA or DNA tests. The results will be of use for the follow-up routines and treatment strategies in Sweden.

Materials and Methods

Our material stems from 15000 women following the normal screening program in the central part of Sweden. All women positive for ASCUS or CIN I with cytology were selected for further studies. All the cytological or histological material was re-evaluated blindly by an experienced pathologist.

The samples positive for ASCUS and CIN I (N=240) were evaluated with PreTect HPV-Proofer (N=240), and a randomised selection of samples was tested by Hybrid Capture II (HCII) and cytology (N=127) and cytology alone (N=112) after 4 months. They were compared with histology from LEEP biopsies (N=126) after 7 months and with PreTect HPV-Proofer (N=240), HCII and cytology after 12 months (Table 1).

All samples with ASCUS and CIN I were tested for mRNA. Colposcopy directed LEEP biopsies (N=126) were taken as a part of the follow-up for all women with an abnormal cytology diagnosis and/or positive HPV DNA test (after 4 months). HPV DNA was detected using the HCII assay (Digene, Gatesburg, MD, USA). Identification and individual typing of E6/E7 mRNA transcripts from HPV 16, 18, 31, 33, and 45 was carried out using the PreTect HPV-Proofer assay (NorChip AS, Klokkearstua, Norway).

Results

The results of HPV tests have been compared with cytology 4 and 12 months after and with histology diagnosis 7 months after positive cytology diagnosis (Table 1). Frequency and distribution of HPV types is presented in table 3. Concordance between cytology and histology was found in 19% of cases (Table 2). Cytology and the DNA test were considerably more often positive in benign and low-grade lesions by histology than the RNA test (Fig. 1). With histology as the "golden standard", the RNA test revealed a higher positive predictive value, and higher specificity (46% and 85,3% respectively) than the DNA test (31% and 51% respectively). However, the DNA test revealed a higher sensitivity (91%) than the RNA based test (81%). 19% of the cases treated with LEEP conization showed aberrant cytology 5 months after treatment (Table 1), 0.5% were found to be CIN II+. HPV DNA was detected in 24% and HPV RNA was detected in 6% of these cases (Table 1).

Discussion and conclusion

The higher positive predictive value and higher specificity of the RNA based method compared with the DNA based method may be explained by the fact that expression of the E6/E7 oncogenes is required for development and maintenance of the malignant phenotype. Frequency of RNA detection is somewhat higher in histological CIN II and lower in histological CIN III

Table 1: Overall results

	0 month	4 month	7 month	12 month
Cytology	240/240 (100%)	93/217 (43%)	Not analysed	30/160 (19%)
HCII	Not analysed	64/113 (57%)	Not analysed	41/169 (24%)
PreTect HPV-Proofer	Not analysed	56/240 (23%)	Not analysed	14/231 (6%)
Histology	Not analysed	Not analysed	100/118 (85%)	Not analysed

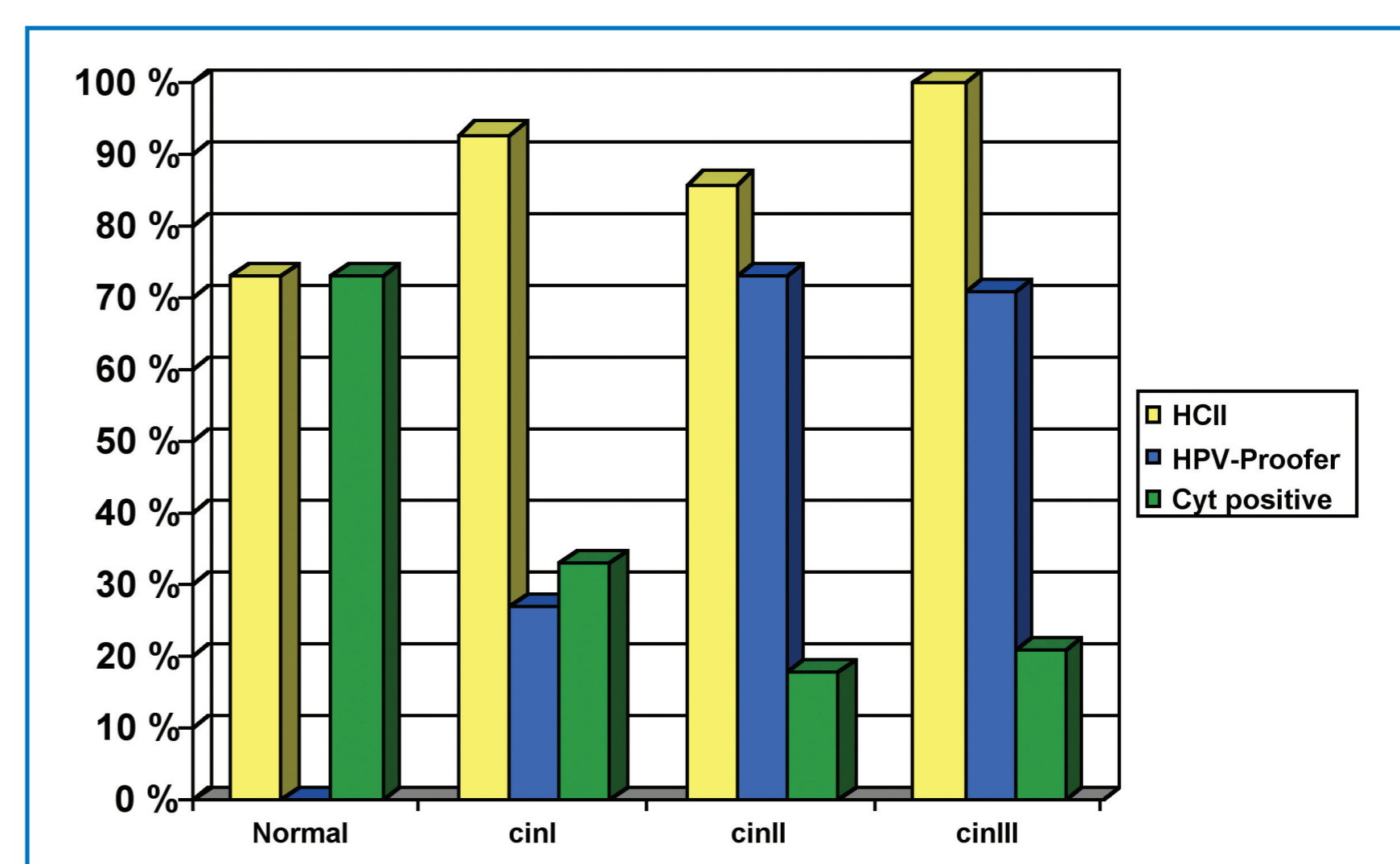
Table 2: Histology versus Cytology 4 months after positive diagnosis.

	Histology							TOTAL
	Normal	Other	CIN I	CIN II	CIN III	Not analysed		
Normal	4	4	9	4	2	104	127	
Inadequate	1	1	1	2	0	4	9	
ASCUS/ASCUS H	9	2	22	9	3/2	4	49/2	
CIN I	4	2	17	3	1	0	27	
CIN II	0	0	1	4	3	0	8	
CIN III	0	0	1	0	3	0	4	
Not analysed	0	0	0	0	0	14	14	
TOTAL	18	9	51	22	14	126	240	

Table 3: Histological results versus HPV DNA, RNA and cytological results

Cytological diagnosis	ASCUS	CIN I	CIN II	CIN III/ASCUS-H
HPV 16	6	4	4	2
HPV 18	2	1	0	3
HPV 31	0	2	1	1
HPV 33	3	2	1	2
HPV 45	3	0	0	1
HPV-Proofer Total	14/49 (29%)	9/27 (33%)	5/8 (63%)	6/6 (100%)
HCII	20/26 (77%)	11/15 (73%)	3/3 (100%)	2/2 (100%)
Histology (CIN II+) only cyt.	3/23 (13%)	3/12 (25%)	4/5 (80%)	3/4 (75%)
Histology (CIN II+) cyt. & HCII	7/20 (35%)	1/11 (9%)	3/3 (100%)	2/2 (100%)
Histology (CIN II+) all samples	12/45 (27%)	4/27 (14%)	7/8 (88%)	5/6 (83%)

Fig 1: Cytology versus HPV test (4 months) and histology (7 months)



than previously seen in a Norwegian study (Kraus *et al.*, in press, JMV 2004). This may be due to differences in evaluation of histology between countries. The risk of missing CIN II+ in women with CIN I or ASCUS, but negative with either HPV RNA or DNA tests was extremely low (0.2%), confirming the added value of HPV testing in cytological ASCUS or CIN I.