

The acetic acid test in evaluation of subclinical genital papillomavirus infection: A comparative study on penoscopy, histopathology, virology and scanning electron microscopy findings

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Abstract

Objectives—To evaluate colposcopic criteria in acetowhite lesions of the penis ("penoscopy") for the diagnosis of subclinical genitoanal papillomavirus infection (GPVI) compared with histopathological criteria of HPV involvement and to various hybridisation assays for HPV DNA detection, and to depict typical lesions by scanning electron microscopy.

Design—The study included 101 randomly selected male partners of females with known GPVI, or with penile symptoms such as itching, burning and dyspareunia who did not exhibit overt genital warts but appeared to be afflicted with acetowhite penile lesions after topical application of 5% aqueous acetic acid. Lesions were judged by penoscopy as either typical, conspicuous or non-typical for underlying HPV infection. Biopsy specimens from 91 men were examined by light microscopy and by either Southern blot (SB), polymerase chain reaction (PCR) and/or in situ hybridisation (ISH) assays for the presence of HPV DNA of the HPV types 6, 11, 16, 18, 31, 33 and 42 (Group A). From another ten men lesions clinically typical for GPVI were also examined topographically by scanning electronic microscopy (Group B).

Setting—The STD out-patient clinic of the Department of Dermatovenereology of Karolinska Hospital, Stockholm, Sweden.

Results—*Group A* Seventy eight (86%) of the biopsied lesions met the penoscopy criteria of being either typical or conspicuous for GPVI. The agreement between penoscopy and histopathology was fairly good, as HPV diagnosis was made by both methods in 56 (62%) of the cases. The reliability of applying strict colposcopic hallmarks was further substantiated by the finding that 55 (60%) of the biopsy specimens taken from penoscopically typical/conspicuous lesions contained HPV DNA. However, there are diagnostic pitfalls for the acetic acid test. Coexistence of an eczematoid reaction with changes indicative of HPV influence

was detected in six (7%) of the cases, while an inflammatory response only occurred in 17 (19%) of the specimens. Additional histopathological diagnoses (normal epithelium, lichen sclerosis et atrophicus, balanitis circinata parakeratotica, verruca plana) were established in another eight (9%) of the cases. Among the HPV DNA positive cases, all of the HPV types tested for were detected with the exception of HPV 18. A severe penile intraepithelial neoplasia (PIN III) was revealed in five (5%) of biopsies; HPV 16 was present in two and HPV 42 in one of these biopsy specimens.

Group B—Scanning electron microscopy depiction harmonised with the penoscopy findings showing that subclinical GPVI characteristically exhibits a well demarcated, slightly elevated border and that the central area of lesions often displays a "groove" in which the epithelium appears to be thin with protrusions from beneath that probably represent capillaries.

Conclusion—Use of the acetic acid test for evaluation of GPVI should be combined with a colposcopic evaluation based on strict topographic hallmarks, followed by a directed biopsy for light microscopic evaluation. We found that the positive predictive value of colposcopy was as high when correlated with histopathological findings (72%) as when virological methods were used, whether HPV DNA hybridisation testing was performed with the well established SB and ISH assays (45%), or by applying the newly introduced and highly sensitive PCR assay as well (71%). False positivity from the acetic acid test occurs and is mainly due to inflammatory conditions but also to the presence of other conditions. Epithelial fissures are evidently associated with some subclinical GPVI lesions and may potentially represent loci minores for infectious stimuli and perhaps facilitate the transmission of some blood-borne STDs. We propose that the term "papillomavirus balanoposthitis" should be used for penile HPV infection associated with inflammatory responses. Our study indicates that PIN

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III frequently occurs in a subclinical form and may be associated with not only previously identified "high-risk" HPV types such as type 16, but also with the HPV type 42 that has not previously been considered as oncogenic.

Introduction

Human papillomaviruses (HPV) are a heterogeneous group of viruses. So far, almost 70 different genotypes have been discovered. Some HPV types exhibit a preferential tropism for mucous membranes, while others rather have predilection for fully keratinized dry skin. The former viruses are harboured mostly in the genitoanal area, but are found in the oro-respiratory mucosa and in the esophageal epithelium as well.¹⁻³

In recent years the use of various hybridisation assays for HPV DNA detection has demonstrated that the predominant majority of individuals with biologically active genitoanal papillomavirus infection (GPVI) is afflicted merely with subclinical flat epithelial lesions that remain invisible to naked eye examination for a considerable time. Evidently the visible genitoanal warts that by tradition have been denominated condylomas (condylomata acuminata) only represent the "tip of the ice-berg".

Some GPVI lesions tend to persist for a considerable time and to be associated with various degrees of dysplastic transformation (intraepithelial neoplasia), in particular of the uterine cervix (cervical intraepithelial neoplasia; CIN), where about 15% of cases progress to carcinoma in situ in time.⁴⁻⁶ The latter type of biological behaviour is associated with "high-risk" HPV types such as HPV 16 and 18,⁷⁻¹¹ as opposed to "low-risk" HPV such as types 6 and 11, which are almost invariably associated with benign epithelial proliferation.^{12,13}

In females it has been shown that flat lesions sometimes are symptomatic, sensations such as itching, burning and/or dyspareunia originating from associated epithelial fissures.¹⁴⁻¹⁶

Although it is of great clinical relevance to recognise correctly the presence of such lesions, no consistently reliable diagnostic methods for routine use have yet been presented for their identification. Some investigators claim that the epithelial whitening that will usually occur from topical application of an aqueous solution of 3-5% acetic acid represents a reasonably accurate method, in particular when combined with magnification by colposcopic equipment.^{13,17-26} Nevertheless, no unequivocally uniform colposcopic criteria have been presented for HPV associated acetowhite lesions. The most consistent pattern appears to be the presence of a well demarcated, slightly elevated acetowhitened epithelium in which a punctuated capillary pattern often can be distinguished.

Histologically, the following morphologic patterns are considered as characteristic of biologically active HPV infection: the presence of an epidermal hyperplasia with acanthotic elongation of the rete ridges, hyper-

parakeratosis, and koilocytosis, associated with the existence of dilated vessels of the corium papillae and some degree of a subepidermal mononuclear inflammatory component. Furthermore, a moderate to severe intraepithelial neoplasia may be seen when lesions are associated with any of the "high-risk" HPV types. Only the presence of koilocytes is pathognomonic of the influence of HPV on the epithelium;^{17,27-33} this hallmark is in general associated with the occurrence of relatively high virus quantities.³⁴ Acetowhite lesions are more frequently void of koilocytosis than is the case for classical condylomas.³⁵

This study focuses on the sensitivity, specificity and predictive value of the colposcopic findings in flat acetowhite lesions of the penis ("penoscopy") as compared with histological criteria of HPV infection and to HPV DNA hybridisation in biopsy tissue. The hybridisation assays performed include the well established Southern blot (SB) and in situ hybridisation (ISH) techniques and also the newly introduced and extremely sensitive polymerase chain reaction (PCR) which may detect as few as 10-100 HPV DNA copies.³⁶ A description of scanning electron microscopic findings in penoscopically typical lesions is also given.

Methods

Patients

One hundred and one randomly selected heterosexual uncircumcised males who attended an STD out-patient clinic because of either penile symptoms such as itching, burning and dyspareunia, or as partners of females with known GPVI without any clinical HPV lesions participated in the study. Aqueous 5% acetic acid was applied to penile epithelium for five minutes by having patients to hold a wetted gauze against the entire penile epithelium, keeping the foreskin retracted.

Penoscopy

Acetowhite lesions were evaluated at magnification (8-20 × 12,5) using a Zeiss photo-colposcope ("penoscopy") and lesions were classified according to the following grading: (1) *Typical*, (fig 1) that is, the presence of well demarcated lesions with a slightly elevated border and the occurrence centrally of punctuated capillaries with or without an associated epithelial depression ("groove"); (2) *Conspicuous*, (fig 2) that is, the presence of well demarcated slightly elevated lesions lacking discernible punctuated capillaries; and (3) *Non-typical*, (fig 3) that is, the presence of lesions exhibiting a ragged border and lacking punctuated capillaries.

Biopsies

Biopsy specimens were obtained subsequent to applying local infiltration anaesthesia using lidocain-HCl 5 mg/ml (Astra, Sweden).

Group A

A specimen about 4-6 mm in diameter was obtained from 91 patients with various peno-

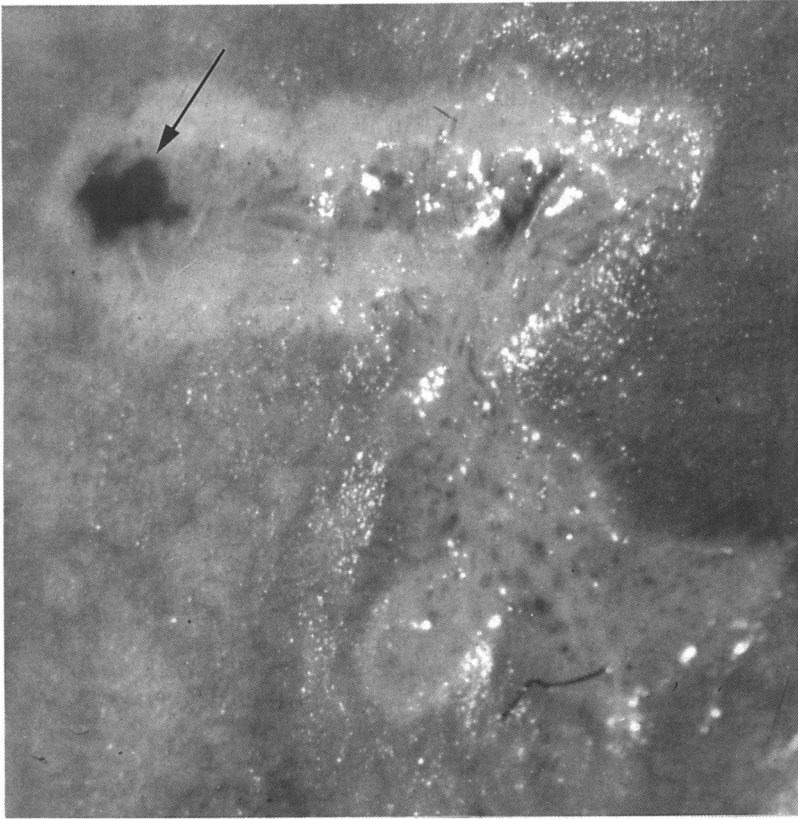


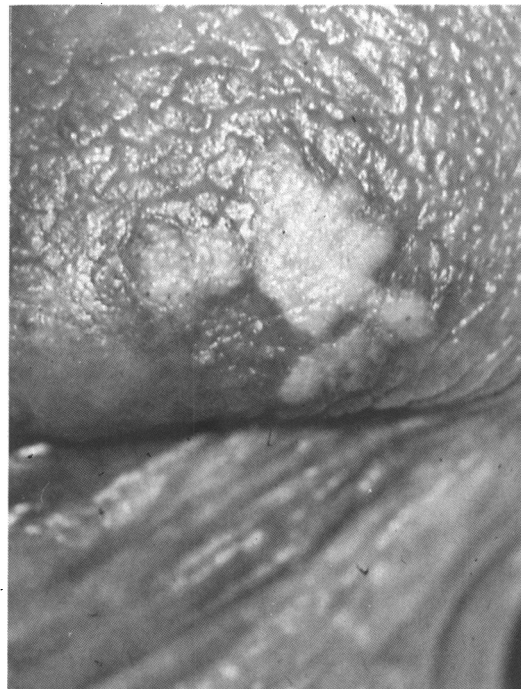
Figure 1 Well demarcated acetowhite lesion on the foreskin. The border is slightly elevated while a central depression ("grove") appears in part of the lesion (top) where a slight haemorrhage is noted (arrow). A punctate capillary pattern is clearly discernable, in particular in the bottom part of the lesion. ($\times 20 \times 12.5$ (Zeiss photocolposcope)).

scopy findings. Half of the specimen was fixed in neutral 10% formalin and paraffin embedded for routine histology and ISH, while the other half was frozen at -70°C for SB and PCR.

Group B

From ten men exhibiting lesions classified as penoscopically typical, a single biopsy was performed that entailed the entire acetowhite area and a 1–2 mm wide zone of adjacent

Figure 2 Well demarcated, slightly elevated acetowhite lesions on the glans penis. The surface is smooth. No punctuated capillaries are seen. ($\times 12 \times 12.5$ (Zeiss photocolposcope)).



epithelium. The specimen was subsequently attached to a piece of cork using pin-point needles, followed by fixation for scanning electron microscopy.

Histology classification

The histological analysis, consistently performed by one of the investigators (MH) without knowledge of either the penoscopy findings or the DNA hybridisation results, was classified according to the following criteria: (1) *Typical*: the presence of epidermal hyperplasia, acanthosis, hyper- and parakeratosis and koilocytosis; (2) *Conspicuous*: all of the criteria above except for koilocytosis; (3) *Conspicuous + eczematoid reaction*: the criteria as in (2) as well as the presence of an eczematous inflammatory response involving the epidermis. (4) *Eczematoid reaction only*; and (5) *Other conditions*. Any intraepithelial neoplasia (PIN) was graded (PIN I-III) according to the criteria defined previously.^{13 37 38}

Hybridisation assays

Owing to relatively small biopsy specimens, all of the various hybridisation methods could not be performed on samples from all of the 91 patients. Tissue sufficient for SB analysis was obtained in 65 males, and PCR could be performed in 61 of the samples. ISH was carried out on 72 specimens. All three assays (SB, PCR, ISH) were carried out in only 27 biopsies but all 91 samples were analysed with at least one of the assays.

Southern blot hybridisation (SB)

(a) *Preparation and restriction cleavage of*



Figure 3 A confluent acetowhite area occurring on the glans penis, in the coronal sulcus and on the foreskin. Some lesions are well demarcated, in other areas the border is somewhat rugged (arrows). No punctuated capillaries are present. Some of the acetowhite in this man occurred on slightly erythematous epithelium, indicating an inflammatory component. ($\times 8 \times 12.5$ (Zeiss photocolposcope)).

DNA The thawed specimens were minced and dissolved at 37°C in lysis buffer containing 50 mM Tris-HCl, pH 7.5, 0.5% SDS, 10 mM EDTA, 50 mM NaCl and 150 µg Proteinase K. After phenol extraction and ethanol precipitation the DNA was pelleted by centrifugation at top speed in an Eppendorf centrifuge for 15–30 min. The pellet was dried and dissolved in 100 µl distilled water. This was further purified by using a GENE CLEAN-kit (BIO 101, California, USA). Restriction enzyme digestion was performed by adding 10 µl 10 × M buffer and 10 µl PstI (Pharmacia LKB, Stockholm, Sweden; 13 000 units) to 80 µl DNA solution, and incubation at 37°C over night.

(b) *Electrophoresis and blotting* Electrophoresis and blotting were performed using a Probe Tech I machine (Oncor Inc., Maryland, USA). The digested samples were supplemented with 10 µl loading buffer (Oncor Inc.) and run according to the manufacturers instructions. The vacuumtransfer was performed for 90 min using Hybond N membranes (Amersham, Buckinghamshire, England). After transfer the membranes were illuminated on a UV light box for 5 min.

(c) *Hybridisation* Cloned genomes of HPV 6, 11, 16, 18, 31 and 42 were used as probes in the hybridisation. HPV 6, 11, 16 and 18 were kindly provided by Prof H zur Hausen, Deutsches Krebsforschungszentrum, Heidelberg, Germany. HPV 31 was obtained from Dr A Lorincz, BRL, Maryland, USA and HPV 42 from Prof G Orth, Institute Pasteur, Paris, France. The complete genomes were cut out and removed from the vector before being labelled. Genome (25–30 ng) was labelled using the Multiprime kit of Amersham Inc. The reaction was performed according to the manufacturers instructions, using either ³²P labelled dATP or dCTP (Amersham; 3000 Ci/mmol) to a specific activity of at least 5 × 10⁸ dpm/µg DNA.

The Hybond membrane was prehybridised in a solution containing 20% deionized formamide 5 × SSC, 50 mM sodium phosphate, pH 6.8, 5 × Denhardt's solution, 0.5% SDS and 250 µl/ml sheared and denatured herring sperm DNA (Boehringer, Mannheim, West Germany) at 42°C for 1–4 h. Low-stringency hybridisation was performed at 42°C over night in a solution of the same composition as used for prehybridisation, except that the sodium phosphate was at 20 mM and herring sperm DNA at 100 µg/ml concentration respectively. Washing was carried out in 2 × SSC, 0.1% SDS, once at room temperature and three times at 52°C. The filter was exposed to X-ray film (Hyperfilm β-MAX; Amersham Inc.) for one to seven days.

In situ hybridisation From formalin fixed and paraffin embedded specimens eight serial sections (5 µm thick) were cut. In situ hybridisation was performed to detect HPV DNA types 6, 11, 16, 18, 31, 33 and 42. One section was hybridised with the vector pBR 322 as a negative control. The HPV probes 6, 11, 16, 18 and 31, 33, 42 were kindly provided from Prof H zur Hausen (Deutsche Krebsforschungszentrum,

Heidelberg, Germany) and Prof G Orth (Pasteur Institute, Paris, France), respectively. The probes were biotinylated with nick translation according to the standard procedures; ISH was done as earlier described with biotin 11-UTP (Sigma).³⁹ Shortly after deparaffinisation and deproteinisation (Proteinase K, 0.5 mg/ml PBS, 15 min at 37°C) hybridisation solution was added onto the sections and denatured in an incubator by heat (90°C, 10 min). The hybridisation solutions contained 50% formamide, 2 × SSC, 10% dextran sulfate, 0.4 mg/ml herring sperm DNA and 1 µg/ml biotinylated HPV DNA probe. The hybridisation was carried out under high stringency conditions for 2 hours at 50°C. Post-hybridisation washes 5 min each included 2 × SSC 0.1% SDS once at RT, 0.2 × SSC, 0.1% SDS three times at 60°C and 2 × SSC once at room temperature. The detection of biotinylated hybrid was performed by sequential incubation in streptavidin, alkaline phosphate (20 min at 37°C) and substrate solution of BCIP (5-bromo-4-chloro-3-indolylphosphate) and NBT (nitroblue tetrazolium) for two hours at 37°C. The reaction was stopped by three washes with water and mounted with aqua mount.

PCR PCR⁴⁰ was performed as described previously⁴¹ using 5 µl of the DNA prepared for Southern blotting. The primers used for HPV 6 were located in the E7 gene at position 563–582 and 698–718 of the genome. The primers used for HPV 16 were localised in the upstream regulatory region at positions 7763–7781 and 61–86 of the genome. The concentration of each primer was 20 pmol per 50 µl reaction volume and the primer pairs for HPV 6 and 16 were run together in the same reaction ("multiplex PCR"). The reactions were performed in an automated thermocycler programmed for 30 cycles of DNA denaturation (95°C), primer annealing (55°C), and template extension (72°C)⁴¹.

Analysis of the PCR product was carried out using 3% NuSieve (FMC BioProducts, Maine, USA) agarose gels containing 0.5 µg/ml of ethidium bromide. As a size standard for the bands on the gel, ΦX 174 DNA restricted with Hae III was used (Pharmacia LKB Biotechnology, Stockholm, Sweden). A band at 156 bp or at 227 bp signified a sample positive for HPV 6 or 16 respectively. To avoid contamination, the PCR was performed in several separate work stations, the reagents were aliquoted from master mixes and the samples were added using piston-driven pipettes (Gilson, France). Solution controls without added DNA were included. High and low positive controls were cloned genomes at 10 pg and 100 fg respectively.

Scanning electron microscopy The biopsies were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide, rinsed in distilled water, dehydrated and critical point dried. The samples were mounted on aluminium stubs, sputtered with gold and viewed in a Philips 400 scanning electron microscope.

Table 1 Correlation between penoscopy and histology findings (%)

Penoscopy	Histopathology		
	HPV concordant influence	Other conditions	Total
Typical/conspicuous	56 (62)	22 (24)	78 (86)
Non-typical	10 (11)	3 (3)	13 (14)
Total	66 (73)	25 (27)	91 (100)

Table 2 HPV DNA hybridisation results (%)

HPV type	Number of positive samples		
	Southern blot (n = 65)	In situ (n = 72)	PCR (n = 61)
6	8	2	26
11	2	0	ND
16	6	2	6
18	0	0	ND
31	1	0	ND
33	ND	1	ND
42	7	7	ND
Unknown	6	ND	ND
Coexistence of more than one HPV type	2	2	12
Total positive	32 (49)	14 (19)	44 (72)

Results

Group A

By penoscopy 91 lesions were assessed, of which 49 (54%) were typical, 29 (32%) conspicuous and 13 (14%) non-typical.

Sixty six (73%) lesions were histologically consistent with GPVI. Of these, 44 (48%) showed koilocytosis, 16 (18%) a conspicuous hyperplasia only, and six (7%) biopsies had an eczematoid component as well. An eczematoid reaction only was detected in 17 (19%) of biopsies. Intraepithelial neoplasia was present in 18 (20%) of lesions; PIN I was detected in three, PIN II in ten and PIN III in another five cases. In three cases the presence of other conditions was established: lichen sclerosus et atrophicus, verruca plana, balanitis circinata parakeratotica in one case each. An apparently normal epithelium was present in five cases.

The correlation between penoscopy and histology findings is shown in table 1. Typical/conspicuous penoscopy findings corresponded to HPV concordant influence in 56 (62%) of biopsies, while 22 (24%) of penoscopically suspected GPVI lesions appeared to represent other conditions. Non-typical penoscopy findings, on the other hand, appeared to be histologically conspicuous for HPV-induced chan-

ges in only 10 cases (11%). This corresponds to a positive predictive value of 72%, a negative predictive value of 23%, a sensitivity of 85% and a specificity of 12% for the penoscopy assessment.

DNA hybridisation data are summarised in table 2. Overall positivity was 49% (32/65) with SB, 19% (14/72) with ISH and 72% (44/61) with PCR.

The correlation between the hybridisation analysis, penoscopy findings and histology results is given in table 3. HPV DNA positivity in typical/conspicuous penoscopy lesions was 49% (29/59) for SB, 14% (9/62) for ISH and 69% (36/52) for PCR. Correspondingly, in lesions that showed light microscopic signs of HPV infection HPV DNA was found in 62% (28/45) with SB, in 28% (14/50) with ISH and in 70% (33/47) with PCR. With either of the hybridisation assays HPV DNA was also detected in lesions considered as non-typical by penoscopy, as well as in biopsies without conspicuous light microscopic signs of viral influence. Thus, non-typical penoscopy lesions harboured HPV DNA in 50% (3/6) with SB, in 50% (5/10) with ISH and in 89% (8/9) with PCR. In biopsy specimens that merely exhibited an eczematoid reaction the corresponding figures were 19% (3/16) and 75% (6/8) for SB and PCR respectively. One case being SB positive for an uncharacterised HPV type revealed a histological pattern characteristic for verruca plana. With PCR this sample was HPV 6 DNA positive. Another biopsy being PCR positive for HPV 6 and 16 was histopathologically typical for balanitis circinata parakeratotica. Three additional samples, apparently representing normal epithelium, contained HPV 6 in two of the cases, and HPV 16 in the third case, when tested with PCR.

All of the three PIN I lesions were HPV DNA positive in the PCR assay, one for HPV 6 and two for HPV 16; one of these lesions was also ISH positive for HPV 42. Of ten PIN II lesions, two were SB positive (one for HPV 6 and the other for HPV 16), one was ISH positive (HPV 6) and eight were PCR positive (four for HPV 6, one for HPV 16 and three for both HPV 6 and 16). Of five PIN III lesions three were SB positive (two for HPV 16 and one for HPV 42); one of the two HPV 16 SB positive cases was also HPV 16 positive in ISH

Table 3 Correlation between HPV DNA hybridisation, penoscopy and histopathology findings (%)

Penoscopy	Hybridisation assay					
	Southern blot (n = 65)		In situ (n = 72)		PCR (n = 61)	
	Positive	Negative	Positive	Negative	Positive	Negative
Typical	15/35 (43)	20/35 (57)	6/41 (15)	35/41 (85)	27/33 (82)	6/33 (18)
Conspicuous	14/24 (58)	10/24 (42)	3/21 (14)	18/21 (86)	9/19 (47)	10/19 (53)
Non-typical	3/6 (50)	3/6 (50)	5/10 (50)	5/10 (50)	8/9 (89)	1/9 (11)
Total	32 (49)	33 (51)	14 (19)	58 (81)	44 (72)	17 (28)
<i>Histopathology</i>						
Typical	19/28 (68)	9/28 (32)	11/35 (31)	24/35 (69)	25/33 (76)	8/33 (24)
Conspicuous	6/12 (50)	6/12 (50)	3/10 (30)	7/10 (70)	6/12 (50)	6/12 (50)
Conspicuous + eczematoid reaction	3/5 (60)	2/5 (40)	0/5 (0)	5/5 (100)	2/2 (100)	0/2 (0)
Eczematoid reaction only	3/16 (19)	13/16 (81)	0/15 (0)	15/15 (100)	6/8 (75)	2/8 (25)
Other conditions	1/4 (25)	3/4 (75)	0/7 (0)	7/7 (100)	5/6 (83)	1/6 (17)
Total	32 (49)	33 (51)	14 (19)	58 (81)	44 (72)	17 (28)

Table 4 Correlation between penoscopy and histology findings versus HPV DNA detection in the various hybridisation assays

Hybridisation assays performed	Total samples analysed	Positive in either test method	Classification of penoscopy and histology findings in HPV DNA positive biopsies			
			Penoscopy		Histology	
			Typical/conspicuous	Non-typical	Typical/conspicuous	Eczematoid reaction and other conditions
SB + ISH + PCR	27	26	25	1	19	7
SB + ISH	24	8	6	2	5	3
SB + PCR	8	6	5	1	5	1
ISH + PCR	21	16	12	4	13	3
SB only	6	6	6		6	
PCR only	5	3	1	2	3	
Total	91	65	55	10	51	14

and another in PCR. A fourth PIN III lesion was PCR positive for HPV 6 only.

Table 4 summarises the correlation between the penoscopy and the histology findings versus the detection of any of the HPV types using the various hybridisation assays. Altogether 71% (65/91) of the biopsies were positive for HPV DNA in either assay. Of these, the penoscopy finding was classified as typical or conspicuous in 55 and as non-typical in another ten cases. The corresponding figures for HPV DNA negative cases were 23 and three, respectively. Thus, typical or conspicuous penoscopy patterns corresponded to a positive and negative predictive value of 71% and 23%, respectively, as evaluated by all of the three hybridisation assays, corresponding to a sensitivity of 85% and a specificity of 12%. If only SB and ISH results are considered, however, the negative and positive predictive values of penoscopy were both 45%, the sensitivity 85% and the specificity 11%.

Analogously, among the 65 HPV DNA positive biopsies 51 exhibited typical or conspicuous light microscopic signs of viral activity while 14 did not. The corresponding figures for HPV DNA negative cases were 15 and 11, respectively. Accordingly, the positive and negative predictive values for the light microscopic criteria were 77% and 44% when all

three virological assays are taken into account, corresponding to sensitivity and specificity values of 78% and 42% respectively. When limiting the analyses to SB and ISH results, the negative and positive predictive values of light microscopy were 59% and 80%, respectively, corresponding to a sensitivity of 88% and a specificity of 44%.

Group B

In the scanning electron microscope the surface of the normal stratum corneum appeared smooth with regular furrows. The analysis of the surface structure of the excised lesions displayed a spectrum of changes. A common feature (fig 4) was the appearance of a distinct, somewhat elevated border between the lesion and the normal stratum corneum with a depressed surface centrally in the lesion. Within the lesion the surface of stratum corneum was smooth in some, while others exhibited a disturbed, "rugged" appearance in which the smooth surface was replaced with a protrusion from beneath (figs 5 and 6).

Discussion

In the present material the tissue collected for light microscopic evaluation was not identical to that analysed for HPV DNA but rather represented adjacent acetowhite epithelium. Furthermore, only one biopsy was collected from each individual, who was usually afflicted with multiple lesions. Because histological features of HPV infection often are focal,⁴² the possibility of sampling error evidently exists. Yet, some relevant results emanate from the present study.

The presence of koilocytosis, reflecting a cytopathic effect of reproductive HPV infection, represents the only pathognomonic light microscopic sign of biological activity.^{17 27-33} This marker correlates best with classical condylomas and is less consistently present in subclinical GPVI.^{35 43} Yet, the presence of koilocytosis in 48% of our biopsies agrees well with a number of previous reports on subclinical lesions.^{15 30 33 44 45} Another 25% of the specimen exhibited less specific structural changes that were highly indicative for active HPV infection. These findings are congruent with the fact that 86% of the lesions were characterised penoscopically as typical or conspicuous for GPVI. Nevertheless, our study clearly demonstrates that differential diagnostic considerations are of major importance when

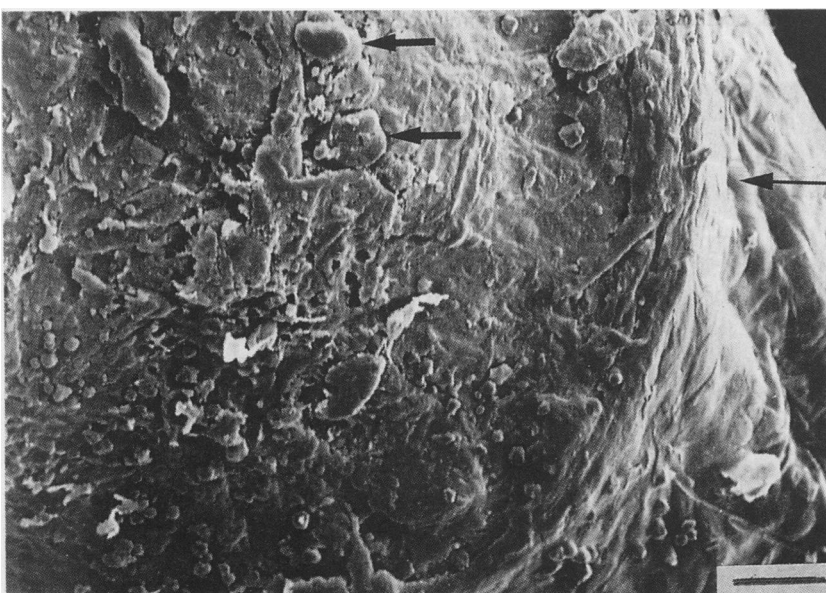


Figure 4 Part of a typical lesion with a marked, slightly elevated border between normal epidermis and the lesion (thin arrow). Within the lesion the surface is rugged with protrusions from beneath (thick arrows). Inset bar = 0.1 mm.

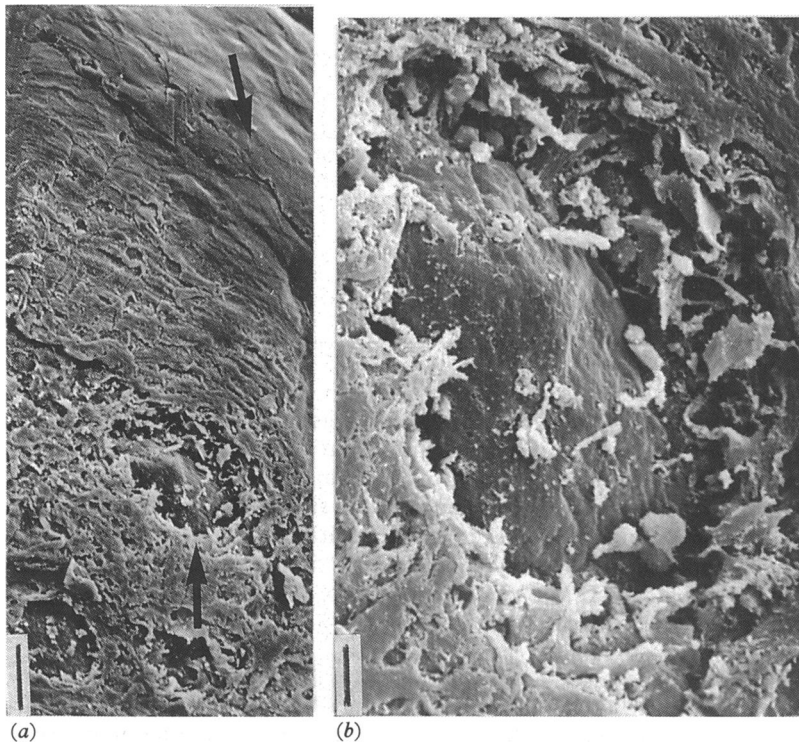


Figure 5 (a) shows part of a lesion with the marked border (upper arrow) and rugged surface with protrusions (lower arrow). Inset bar = 0.05 mm. (b) shows the protrusion in figure 5a at a higher magnification. Inset bar = 0.01 mm.

acetowhite genital lesions are detected.

Inflammatory conditions such as balanoposthitis as the cause of acetowhiting has been considered also previously.^{19,46} In 7% of our cases inflammatory cell infiltrates coexisted with conspicuous HPV changes. The existence of merely an eczematoid reaction was found in 19% of the biopsy specimens; in these cases HPV DNA was detected in 19% by SB and in 75% by PCR. These observations indicate that the concurrent presence of GPVI and local inflammation is not uncommon. This concept also agrees with the high frequency (50–89%) of HPV DNA positivity that was detected in lesions penoscopically classified as non-typical; in many of these cases the acetowhiting occurred on an erythematous epithelium. An

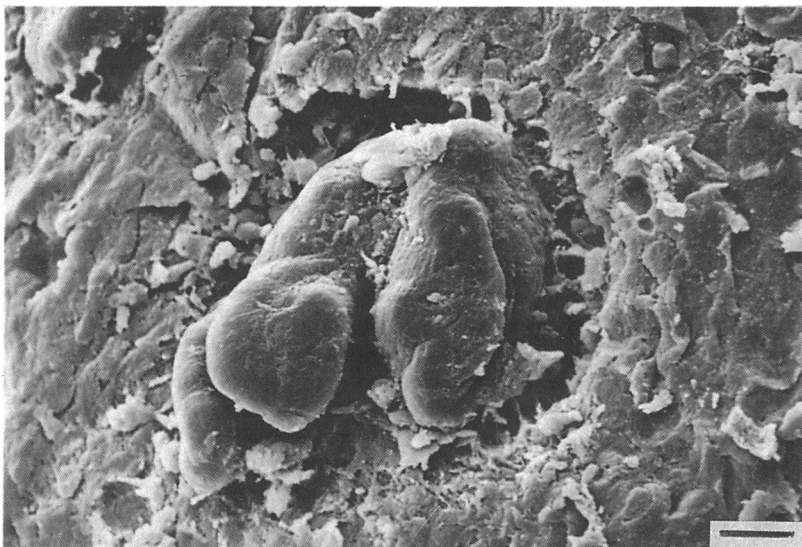


Figure 6 Part of a typical lesion with a nodular protrusion from beneath through the surface of the lesion. Inset bar = 0.015 mm.

association between HPV influence and inflammation has also been substantiated by Arumainayam *et al.*,⁴⁷ who found that koilocytotic changes were present in 49 of 50 biopsy specimens taken from men presenting with a patchy balanoposthitis.

As is apparent by penoscopy (fig 1) as well as scanning electron microscopy (fig 4) some acetowhite lesions exhibit a central groove in which the epidermis apparently is quite thin. It seems reasonable to believe that associated epithelial fissures represent loci minores for the entrance of exogenous toxic and/or infectious stimuli that may initiate an inflammatory response. These epithelial breaks may possibly also facilitate the transmission of blood-borne agents such as syphilis, HBV (hepatitis B virus) and HIV (human immunodeficiency virus). This concept harmonises well with the scanning electron microscopic findings, indicating that capillaries protrude through the epidermis in some of the lesions. Symptoms such as itching, burning or dyspareunia have been associated with GPVI on the female genitals and have been denominated as “papillomavirus vulvovaginitis” and “pruritic vulvar squamous papillomatosis”.^{14,48–51} We propose the term “papillomavirus balanoposthitis” for corresponding illness in males, and we suggest that recurrent balanoposthitis and vulvovaginitis of otherwise unknown aetiology should be investigated for the potential of an underlying GPVI.

In a subsequent pilot study on 26 uncircumcised males presenting themselves with various penile symptoms such as itching, burning and/or dyspareunia, and in whom acetowhite lesions were detected in the preputial cavity, we found that topical anti-inflammatory therapy for two weeks resulted in a complete or partial clearing of the acetowhiting in two thirds of the patients (von Krogh & Wikström, unpublished). Lesions that most consistently responded to anti-inflammatory therapy in general exhibited a ragged, irregular border and a varying degree of adjacent erythema. However, in a number of cases such lesions concurred with sharply demarcated lesions that we considered as conspicuous for GPVI. Further evaluation of these findings is required, however, to ascertain the potential coexistence of penile HPV lesions with balanoposthitis.

An inflammatory response in close vicinity to HPV induced epithelial changes may also represent a secondary immunological response. It is well appreciated that spontaneous wart rejection is associated with a dermal accumulation of lymphocytes and macrophages that subsequently penetrate into the epidermis, causing an eczematoid reaction similar to that observed in a quarter of our patients.^{52–54} A phenotypic characterisation of the inflammatory cells deserves further attention in future studies on such patients. Also, the natural course of subclinical GPVI in men should be investigated; a marked focal inflammatory response might very well be associated with a favourable outcome. Interestingly, of lesions that histologically showed the mere

existence of inflammation, HPV DNA was detected in no sample analysed with ISH but in 19% (3/16) of specimens investigated with SB and as much as 75% (6/8) of those examined with PCR. Owing to the differences in sensitivity for these assays, these findings may very well reflect that a relatively low HPV DNA copy number exists in lesions being in the phase of involution.

Several screening investigations have demonstrated that subclinical GPVI is quite common in sexually active individuals but that the type specific HPV carriage rate differs from that of condylomata patients.⁵⁵ Using dot-blot hybridisation on exfoliated cervical cells, Soares *et al.*,⁵⁶ detected HPV 6/11 in 4–7% as compared with positivity rates of 17–29% for HPV 16/18, of 18–24% for HPV 31/33/35 and of 51% for other uncharacterised HPV types. Using the more sensitive SB assay on cervical smears Rosenfeld *et al.*⁵⁷ detected HPV 6/11 in 6% of the positive samples as compared with HPV 16 in 16%, HPV 18 in 15% and an uncharacterised HPV type in another 67% of cases. These results are congruent with previous reports on biopsy specimens sampled from penoscopically identified lesions of males. Thus, Barrasso *et al.*¹³ found that condylomas in general were associated with HPV 6, 11 or 42, while subclinical lesions predominantly were associated with the HPV types 16, 18 or 33. In our material, however, the type specific HPV distribution was somewhat different with a relatively high frequency of HPV 6/11 that amounted to 31% with SB, to 14% with ISH and to 59% with PCR (table 2). We did not detect the presence of HPV 18 in any case, a finding corresponding to previous investigations on penile warts in our geographical area indicating that HPV 18 infections are rare.⁵⁸ As accounted for in previous studies concurrent infection with more than one HPV type was not uncommon.^{58,59}

The presence of a penile lesion being histopathologically characteristic of *verruca plana* has not been reported previously. The HPV DNA detected in SB did not hybridise with any of the probes used by us but possibly correspond to a genome related to HPV types 3, 10 or 28.⁶⁰

As demonstrated by Barrasso *et al.*¹³ aceto-white subclinical penile lesions may reveal the presence of "bowenoid" epithelial changes (PIN III), indicating infection with oncogenic HPV types, in particular HPV 16. PIN III was found in five of our specimens. As merely a single biopsy specimen was collected for light microscopy in our material, the potential of sampling errors entails that the prevalence of PIN might be considerably higher in sexually active males than found in the present study. We detected HPV 16 DNA in two of the PIN III lesions with either of the hybridisation assays, while one additional sample harboured HPV 42 as revealed by SB. While originally isolated from a case of CIN⁶¹ HPV 42 has subsequently been associated with benign penile lesions¹³ and has not been considered an oncogenic HPV type.⁶² However, our results indicating that HPV 42 may possess an

oncogenic potential, deserve further attention.

For subclinical GPVI no single diagnostic "gold standard" exists. Acetowhite mucosal changes comprise a spectrum of colposcopic and histological abnormalities. Accordingly, the clinician and the pathologist will often encounter lesions that do not unequivocally fulfill all of the criteria for GPVI. The use of ISH for HPV DNA detection has proved valuable for increasing the accuracy of light microscopic evaluation and has been recommended as a quality control procedure for the histological diagnosis of HPV-related lesions.⁶³ Still, we feel that a combined appraisal of clinical presentation, use of the acetic acid test combined with colposcopic evaluation of lesional topography, and application of conventional histology criteria will be satisfactory in the predominant number of routine cases. The potential value of using virological methods in clinical routine has been the cause of much controversy recently,^{64,65} in particular with regard to the PCR methodology that possibly may be able to pick up latent infections as well.³⁶ HPV was detected by the highly sensitive PCR assay in as much as 69% of typical/conspicuous penoscopy lesions and 70% of biopsies being light microscopic typical/conspicuous of viral influence but also in 60% of samples that were histologically normal. Accordingly, the relevance of applying the PCR method in the analysis of our study is unclear. Nonetheless, the addition of virological methods to conventional histopathology did not improve the diagnostic accuracy of our penoscopy findings. Thus, the positive predictive value between penoscopy and light microscopy (72%) is higher than the corresponding value for virological methods whether calculated on the basis of SB and ISH results alone (45%), or applying PCR results as well (71%).

The startling flow of epidemiological data presented during the past few years, revealing that latent or subclinical GPVI is more than 100 times as common as manifest wart disease,⁵⁵ has created some ambiguity among clinicians on the optimal management of patients with GPVI. It has been suggested that women with CIN may benefit from examination and treatment of subclinical lesions of their male partners, aiming at optimising the cure rates of CIN. However, it has been shown that the treatment failure rate of women with CIN, whose partners are carefully treated, does not differ from that of women whose partners remain untreated.⁶⁶ Accordingly, routine screening with the acetic acid test in males whose partners have CIN does not rely on a somatic rationale. In the light of available epidemiological data it rather seems warranted to discourage a use of the acetic acid test for screening purposes, as an ill considered use of the test may create unnecessary fear and anguish in presumably healthy individuals. It rather seems rational to await a spontaneous resolution of asymptomatic subclinical GPVI of the outer genitals. Although current knowledge on the natural course of such lesions is quite limited, studies on cervical lesions

indicate that the majority of cases will clear spontaneously within a period of 5–6 years.^{5 6 7} Age related prevalence studies on HPV carriage are in agreement with this concept.^{56 58} It has also been demonstrated that bowenoid lesions of the outer genitals appear to regress spontaneously in up to 75% when observed for a period of 1–2 years.⁵²

Patient management must rather rely upon pragmatic approaches of diagnosing and treating clinically relevant disease. This approach entails eradication of condylomas and overt bowenoid papulosis, of moderate to severe CIN, as well as of acetowhite lesions if these are associated with any symptoms. For this purpose it is important that physicians are familiar with the colposcopic criteria that indicate underlying GPVI and also are aware that false positivity from the acetic acid test may arise from other conditions such as lichen sclerosus et atrophicus and, above all, of inflammatory conditions.

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