Papillomavirus-associated balanoposthitis

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Abstract

Objective—To assess whether there might be an association between genital papillomavirus infection (GPVI) and balanoposthitis.

Design—Retrospective HPV DNA examination of biopsy specimens from 23 men suffering from balanoposthitis and exhibiting acetowhite lesions that were penoscopically and histologically concurrent with HPV infection.

Setting—The STD clinics at Karolinska Hospital and South Hospital, Stockholm, Sweden.

Participants—Randomly selected men attending with long-lasting and/or recurrent penile symptoms and exhibiting a clinical picture of balanoposthitis, who revealed a penoscopical and histopathological picture of epidermal lesions that were concordant with accepted criteria for typical or conspicuous GPVI. Asymptomatic controls were selected retrospectively on the basis of identical penoscopy and histology criteria.

Results—A history of previous condylomata was obtained in eight (35%) of 23 men. At penoscopic evaluation tiny condylomatous lesions were observed in five (22%) patients. The *in situ* hybridisation (ISH) assay using specific probes for the HPV types 6/11, 16/18, 31/33 and 42 was positive in 13/23 (56%) of the patient samples, but in only 26% of the 19 control samples. In patient biopsies the oncogenic HPV types 16/18 and/or 31/33 were found in 7/13 samples, whereas HPV 6/11 and/or 42 were present in another six cases. PCR performed on the ten ISH negative patient biopsies, were negative in all cases.

Conclusion—Symptoms included redness, itching, burning, tenderness, dyspareunia, fissuring and in two cases penile oedema and inguinal adenopathy. All patients fulfilled penoscopical and histopathological criteria for HPV infection. We demonstrate some tentative evidence that HPV might be associated with long-lasting balanoposthitis, although our data still are circumstantial for a causative association. The results also elucidate the diversity in clinical presentation of GPVI.

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Introduction

Altogether 28 HPV genotypes exhibit a pref-

erential tropism for the genitoanal area (genitoanal papillomavirus infection; GPVI).¹ The typical condylomata acuminata (genital warts), known since ancient times,² appear to be predominantly caused by the HPV types 6 and 11, which are at present classified as "low risk" HPV types owing to their lack of significant oncogenic potential.³ However, these warts merely represent the "tip of the iceberg"^{4,5}; GPVI most often gives rise to lesions that are undetectable by naked eye examination unless visualised by application of an aqueous solution of 3-5% acetic acid.⁶⁻⁸

While some acetowhite lesions are associated with HPV 6 and 11 or with other "low risk" types such as HPV 42, others are induced by potentially oncogenic "high risk" types such as HPV 16, 18, 31 and 33.^{7,8} The relative proportion of low versus high risk HPV types in acetowhite lesions varies in different studies.^{6,7}

Varying degrees of dysplastic epithelial transformation, referred to as intraepithelial neoplasia, commonly occur in the genital area through HPV influence. The cervix uteri represents a locus minoris for the development of persistent and progressive dysplasia (cervical intraepithelial neoplasia; CIN) with a relatively high potential for progression into invasive cancer when associated with oncogenic HPV types.^{9,10}

Most research on males afflicted with acetowhite penile lesions has focused on the potential role of the males representing vectors for spread of the infection to the female population,^{7,11-18} while less attention has been given to the potential morbidity for the male counterpart. Although acetowhite penile lesions apparently mostly are asymptomatic, they may in some cases give rise to symptoms such as itching, burning and dyspareunia.⁶

Recently, diagnostic criteria for typical and conspicuous acetowhite penile lesions, as evaluated by colposcopic magnification ("penoscopy"), have been discussed in detail.⁶ Yet histopathology evaluation appears to represent the "gold standard" for a final verification of underlying GPVI in an acetowhite lesion. Benign epidermal hyperplasia and various degrees of intraepithelial neoplasia are parts of a continuous morphological spectrum.^{19,20} A pathognomonic histology entails the presence of an acanthotic epidermis with the occurrence of koilocytosis in the stratum spinosum. Additional conspicuous criteria entail parakeratosis and dilated dermal capillaries. The existence of intraepithelial neoplasia is common, and may or may not be associated with koilocytosis. Intraepithelial neoplasia on the outer genitals in young adults is almost

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Table 1 Symptoms and signs in 23 men exhibiting penoscopic findings indicating HPV infection

Symptoms		Signs	
burning	12 (52%)	erythema	21 (91%)
fissuring	10 (43%)	fissuring	8 (35%)
redness	9 (39%)	oedema	1 (4%)
discomfort during intercourse	9 (39%)	inguinal adenopathy	1 (4%)
tenderness	6 (26%)		
itching	5 (22%)		
penile swelling	1 (4%)		
inguinal pain	1 (4%)		

unequivocally associated with HPV infection; while low risk HPV types occasionally are associated with mild to moderate dysplastic changes, severe dysplasia strongly indicates the presence of high risk HPV types.²⁰⁻²²

Available HPV DNA hybridisation assays are of significant investigative relevance for type-specific HPV detection but differ regarding sensitivity, specificity and applicability.²³ The particular benefit of the in situ hybridisation (ISH) technique is that a correlation can

be made between presence of HPV DNA and underlying histopathological morphology.²³ However, although highly specific, the sensitivity of ISH is relatively low, allowing a detection of > 10 virions per cell.²⁴ The polymerase chain reaction (PCR) is the most sensitive assay, as the amplification allows one specific viral genome to be detected among 100 000 cells.²⁵ However, sensitivity may be influenced by the type(s) of primer(s) and technical handling.²⁶

In the present study we have analysed retrospectively biopsy specimens taken from either penoscopically typical or conspicuous acetowhite lesions of 23 men attending due to long-lasting and/or recurrent penile symptoms. All samples were investigated using conventional light microscopy and in situ hybridisation; ISH negative cases were further analysed with HPV consensus PCR primers. Specimens collected from asymptomatic men afflicted with penile acetowhite lesions exhibiting equivalent penoscopic signs, were considered as controls and were investigated with ISH.

Materials and methods

Patients The study includes 23 randomly selected symptomatic uncircumcised heterosexual men examined at the STD clinics at the Karolinska Hospital and at the South Hospital in Stockholm, Sweden, during a four-year period (1987-1991); all of the men suffered from long-lasting and/or recurrent penile itching, burning, tenderness, dyspareunia, oedema and/or inguinal pain (table 1). Clinical signs entailed varying degrees of macroscopically apparent balanoposthitis including erythema, and in some cases the occurrence of fissures, oedema and/or inguinal adenopathy as well. Urethral specimens were collected for direct microscopy (methylene blue staining) at high-power view (× 1000), and for cultures of *Neisseria gonorrhoeae* and for ELISA assay detection of *Chlamydia trachomatis* (Syva Scandinavia, Stockholm, Sweden). Concurrent syphilis was ruled out by the standard VDRL test, and HIV infection by ELISA technique (Abbott Scandinavia, Stockholm, Sweden). According to current clinical routine in our setting, all men exhibiting signs of balanoposthitis were prescribed topical anti-inflammatory therapy using Daktacort[®] cream (miconazol and hydrocortison; Janssen Pharmaceutica, Belgium) twice daily for two weeks. The present study only includes men who followed these instructions and who subsequently promptly returned, and in whom persistent acetowhite lesions were detected at reevaluation at this time. Persistent acetowhite lesions were classified penoscopically as being either typical or conspicuous of GPVI. Lesions were classified as typical when they exhibited a well demarcated, slightly elevated border as well as discernible punctuated capillaries, while lesions were categorised as conspicuous if they displayed identical criteria except for the presence of punctuated capillaries.⁶

Figure 1 21 year old male with no history of previous STD. During a 4-months period noted erythematous patches in the sulcus coronarius and periodically experienced some dyspareunia associated with tenderness of the afflicted area. Penoscopy revealed maculopapulous erythematous lesions some of which exhibiting typical punctuated capillaries (a; arrow), being sharply demarcated using the acetic acid. The biopsy showed a picture of PIN II (b), but no HPV DNA was detected in the ISH assay. Diathermy performed at three occasions during a 3-months period, thereafter free from symptoms during a 6 months follow-up period.

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Table 2 Result of the *in situ* hybridisation (ISH) assay with type-specific HPV probes in 23 biopsies

HPV 6/11	2 (9%)
HPV 16/18	4 (17%)
HPV 31/33	3 (13%)
HPV 42	3 (13%)
HPV 6/11 + 42	1 (4%)
Total	13 (56%)

Controls As controls, 19 asymptomatic men were retrospectively selected among biopsy samples previously collected from 91 men presenting with penoscopically typical or conspicuous acetowhite penile lesions⁶ exhibiting histopathological signs typical for GPVI, that is, the presence of koilocytosis and/or PIN. These samples were analysed with ISH. The presence of concurrent STDs and urethritis was tested analogously to that of the patients.

Biopsies One biopsy specimen was taken from each patient after local anaesthesia with lidocaine-HCl 5 mg/ml (Astra, Sweden). In the patient group biopsies were generally somewhat larger (4–6 mm) compared with those of the controls (2–3 mm).

Light microscopic evaluation The specimens were fixed in neutral 10% formalin and paraffin embedded for routine histology. All were examined by the same dermatohistopathologist (M.H.). Specimens showing epidermal hyperplasia, acanthosis, parakeratosis and koilocytosis were classified as typical, whereas those exhibiting all criteria except for koilocytosis were classified as histopathologically conspicuous, unless they displayed any sign of intraepithelial neoplasia (PIN), when they were also classified as typical. Any PIN was further graded as degree I, II or III, based on accepted criteria entailing the extent of dysplastic involvement of the epithelium.^{7,27} Any dermal inflammatory infiltrate was graded as being mild, moderate or severe. Fifteen biopsies were also PAS stained for signs of potentially persistent fungal infection.

Hybridisation assays Paraffin-embedded biopsy specimens from all of the patients were examined with ISH, and in ISH negative samples also with PCR; ISH was performed on the control biopsies.

ISH *In situ* hybridisation was performed to detect HPV DNA specific for the HPV types 6/11, 16/18, 31/33 and 42 using a biotinylated whole genomic probe as earlier described.^{24,28} Shortly, after deparaffinisation and deproteinisation, the sections were simultaneously denatured with the hybridisation mixture containing 2 µg/ml biotinylated probe in 2 × SSC, 50% formamide, 0.4 mg/ml herring sperm DNA, 10% dextran sulphate. After overnight hybridisation at 55°C, the sections were subsequently washed with 2 × SSC at room temperature, 0.2 × SSC at 60°C and 2 × SSC at room temperature. The hybrids were detected by streptavidin-alkaline phosphatase (Amersham International plc, Amersham, UK) complex using NBT and BCIP as chromagen.

PCR One to several 5-µm thick sections were cut to achieve an average surface area of 1 cm.² Before slicing, the excess paraffin was cut off from the block. The sections were directly placed into a 500-µl microfuge tube. The paraffin was extracted twice with 500 µl xylene by mixing gently for 5 minutes at room temperature, followed by washes with absolute ethanol to remove xylene. After

removing the ethanol, a few drops of acetone was added to each tube to be evaporated. On to dried samples, 50 µl of sterile distilled water was added. The pellet was gently resuspended and boiled for ten minutes. The tubes were transferred on ice and centrifuged to pellet the undissolved material. Samples were used immediately or stored at –20°C until used. For PCR, 15 µl of supernatant was used. PCR was done in 50 µl of the reaction mixture (50 mM KCl, 10 mM TrisHCl (pH 8.8), 1.5 mM MgCl₂, 0.1% Triton X-100), 20 pmol of primers, 200 mU of each deoxynucleotide triphosphate and 0.75 units of DynaZyme DNA polymerase (Finnzymes, Espo, Finland).

For the detection of HPV DNA the consensus primers from the L1 region were used as described by Manos *et al* in 1989.²⁹ The specificity of the amplified PCR product was confirmed by digestion with the restriction enzymes Pst I and Rsa I (BioLabs).

Results

Patients The mean age of the men was 29 (range 19–58) years; median age was 26 years. No case of urethritis was detected, and all men had negative tests for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, syphilis and HIV. A history of previous condylomata was reported by eight (35%) of the men.

Penoscopically typical GPVI lesions were detected in 10 (43%), and conspicuous changes in another 13 (57%) of the 23 men.

Penile symptoms included burning in 12 (52%), redness in nine (39%), some degree of discomfort during intercourse in nine (39%), tenderness in six (26%) and itching in five (22%) of the men (table 1). Ten (43%) of the men complained about epithelial fissures that had periodically been painful to some degree and frequently given rise to periodic moderate dyspareunia. One man (4%) had experienced a sudden onset of penile swelling that lasted for some days. Another patient reported recurrent inguinal pain for about a month. Exact information about the duration of symptoms was available in 18 patients; the mean was 11 and the median four (range 0–48) months.

Clinical investigation demonstrated a localised and partial erythema of the glans, the sulcus coronarius, the fraenum and/or the foreskin in 21 (91%) of the men, while eight men (35%) exhibited a penoscopically apparent fissuring (table 1). One man (4%) presented with a marked penile oedema (fig 2a). Another patient had been referred to the department because of unexplainable inguinal adenopathy that was confirmed by us at attendance. Penoscopy revealed that five of the men (22%) were concurrently afflicted with tiny acuminate or papular lesions that were inapparent by naked eye examination. Only one of these five men had a history of previous condylomata.

Altogether 21 (91%) of the 23 biopsy samples showed light microscopic typical features of HPV infection; koilocytosis was detected in 15 (71%) of the typical samples.

The histology was classified as conspicuous in another two cases (9%). PIN was noted in 17 of the biopsies (74%); PIN I occurred in six (26%), PIN II in seven (30%) and PIN III in four (17%) samples. Of samples exhibiting PIN, 11/17 (65%) exhibited focal areas of koilocytosis, occurring either in direct association with dysplasia, or occurring in adjacent areas of the samples. Various degrees of dermal lymphocytic inflammatory infiltrates were present in 17 (74%) of the cases; mild, moderate and severe in seven (30%), eight (35%) and two (9%) biopsies, respectively.

ISH was positive in 13 (56%) of the 23 samples, two being positive for HPV 6/11, four for HPV 16/18, three for HPV 31/33 and three for HPV 42. Double infection with HPV 6/11 and 42 was present in one case (table 2). Of samples that were histopathologically typical for HPV infection, 11/21 (52%) were HPV positive. The ISH was also positive in both of

the two samples histologically classified as conspicuous. The PCR assay was performed on ten ISH negative biopsies, in all cases with a negative result. Candida was not detected in any of 15 PAS stained samples.

In table 3, penoscopy, histopathology and ISH findings among the 23 men included in the study are summarised. Penoscopically, a conspicuous picture was found in 41% and a typical appearance in 59% of the PIN cases.

Controls The mean age, 25 years (range 20–31 years), was somewhat lower than that of the patients; median age was 24 years. No cases of urethritis or other STDs were diagnosed among the controls. These men neither presented themselves with symptoms, nor had a history of or clinical signs of balanoposthitis. However, a history of previous condylomata was reported by 15 (79%) of the men.

Penoscopically, 12 (63%) of the men exhibited typical and seven (37%) conspicuous acetowhite lesions. Furthermore, by penoscopy four (21%) of the men appeared to be concurrently afflicted with tiny acuminate or papular warts.

Histopathological examination showed typical HPV signs in all of the controls; koilocytosis was present in 15 (79%) and some degree of PIN in 12 (63%) samples. PIN I, II and III was detected in three (16%), eight (42%) and one (5%) of the biopsies, respectively. Of biopsies revealing any PIN, 8/12 (67%) concurrently exhibited focal areas of koilocytosis. Dermal inflammatory infiltrates were present in 17 (89%) samples, graded as mild, moderate and severe in eight (42%), seven (37%) and two (10%) biopsies, respectively.

The ISH assay was positive in 5/19 (26%) cases, with HPV 16 present in one (5%) and HPV 42 in four (21%) of the biopsy specimens.

Discussion

Although no classical condylomas had been observed by the patients at the time of investigation, as much as 35% of the patients and 79% of the controls gave a positive history of previous condylomata therapy. Furthermore, tiny acuminate and/or papular warty lesions, unnoticed by the patients, were detected by penoscopy in 22% and 21% of patients and controls, respectively. As the present study is based on penoscopic criteria that previously have demonstrated a high predictive value for the detection of acetowhite GPVI lesions,⁶ and all 23 patient biopsies revealed one or more histological signs of HPV influence, we are inclined to believe that the present material represents true cases of biologically active HPV-associated lesions. This premise is further underscored by the fact that koilocytosis was present in 15 (65%) and some degree of PIN in 17 (74%) of the 23 patient biopsies. In this respect, corresponding figures among the controls were highly congruent, with koilocytosis and/or PIN detected in 79% and 63%, respectively. Of the 17 patient biopsies exhibiting any degree of PIN, 11 (65%) also exhibited concurrent koilocytosis; the corresponding figure for controls was 8 of 12

Figure 2 24 year old subjectively healthy male with no history of previous STD attended because his steady female partner was diagnosed with GPVI. No warts were found but he was treated with diathermy due to presence of minute penoscopically typical GPVI lesions in the sulcus coronarius. At a 2-months follow-up still subjectively healthy, the acetic acid test being negative and penoscopy evaluation indicating normal conditions. After another two months a sudden onset of oedema afflicting the foreskin and the distal part of the penile shaft (a). After retraction of the foreskin and performance of the acetic acid test a conspicuous acetowhite lesion appears (b; arrow). Genital examination otherwise normal. Treated orally with norfloxacin and with topical anti-inflammatory therapy. Within a week complete disappearance of oedema. Thereafter free from symptoms during a 2-years follow-up period. This male harboured HPV 42, and the biopsy exhibited PIN grade I.

Table 3 Summary of the results of penoscopy, histopathology and in situ hybridisation (ISH) for 23 men

Patient number	Age (years)	Duration of symptoms (months)	Previous condylomas	Penoscopy			Histopathology					
				Typical	Conspicuous	Concurrent microcondylomas	Typical	Conspicuous	Koilocytosis	PIN	Dermal inflammation	ISH
1	21	4		x			x		x	II	—	—
2	33	4	x	x			x			I	+	—
3	24	6			x	x	x		x	I	—	6/11 + 42
4	24	0	x				x		x	I	—	42
5	25	1			x	x	x		x	0	—	42
6	45	1			x			x		0	++	6/11
7	26	?	x		x		x		x	II	+++	31/33
8	31	3			x		x		x	0	+++	31/33
9	26	6			x		x		x	0	+	6/11
10	24	48	x	x			x		x	III	—	—
11	27	24	x		x	x	x			I	++	—
12	27	3			x		x		x	0	+	—
13	29	2			x		x		x	I	+	—
14	58	36			x			x		0	++	42
15	22	1		x		x	x			III	+	—
16	24	?	x	x			x		x	I	++	—
17	28	6			x		x		x	II	++	—
18	27	?	x	x			x		x	III	—	16/18
19	45	24		x			x			II	+	31/33
20	24	2		x		x	x			II	++	16/18
21	27	?	x	x			x			II	+	—
22	26	12			x		x		x	III	++	16/18
23	19	?		x			x			II	++	16/18

PIN:Penile intraepithelial neoplasia is graded as 0 if absent, and as degree I (mild), II (moderate) or III (severe).
Dermal inflammation: If present, the reaction is graded as mild (+), moderate (++) or severe (+++).

(67%). In our investigation we also have classified six patient biopsies exhibiting PIN without concurrent koilocytosis (35%) as being light microscopically typical for GPVI. The validity of considering PIN I as an indicative criterion of underlying HPV infection might possibly have been deceptive if anti-inflammatory pre-treatment had not been given consequently in our patients. Yet, in two thirds (4/6; table 3) of biopsies exhibiting PIN I, koilocytosis was also detected; therefore, we do believe that a mild dysplasia in penile epidermis represents a reliable sign of HPV infection.

The fact that positive ISH signals were detected in merely 56% of the patient biopsies, and at a frequency as low as 26% in control biopsies may seem confounding, as all ISH negative biopsies had been classified as typical for GPVI by penoscopy as well as by histology evaluation. Accordingly, it seems likely that either suboptimal ISH test conditions existed, or that some other HPV type(s) than the ones being tested for potentially were pathogenetically involved. Regarding ISH

sensitivity rates, biopsy sample size is of importance, as one area of the lesion could contain HPV DNA and another not.²⁵ The fact that we analysed relatively small samples may have been of major significance. Thus the ISH positivity rate was higher in patient samples (4–6 mm) than in control biopsies (2–3 mm). Using L1 derived consensus primers, PCR positivity did not occur in any of our ISH negative patient biopsies. It is possible that the use of other types of consensus primers,²⁶ or of nested PCR primers³⁰ might have given different results. However, in future studies on patient categories similar to the one studied by us, we recommend a prospective design and the combined use of PCR and Southern blot assays.

The ISH positivity rate of 56% in our study falls within the lower range of 59–84% accounted for in various studies using analogous methodology applied on samples from clinically and/or histologically typical/conspicuous GPVI lesions.^{20 21 31} However, ISH positivity also correlates with the degree of intraepithelial neoplasia. In general, a positive ISH correlation tends to exist with the presence of koilocytosis, while lower ISH positivity rates often are associated with lesions merely suggestive for HPV infection such as nuclear enlargements³² and intraepithelial neoplasia.³¹

In a previous ISH study by Barrasso *et al*,⁷ 12/20 (60%) of acetowhite macular lesions harboured low risk HPV types, while 8/20 (40%) were positive for oncogenic types. On the other hand, in a similar investigation⁶ on subclinical penile lesions we found that of 32 HPV DNA positive biopsies, as much as 17/32 (53%) were associated with low risk types, only 7/32 (22%) with oncogenic types, and uncharacterised HPV types in the remaining HPV DNA positive cases. The rather even distribution of benign versus oncogenic HPV types in HPV positive samples in the present series is somewhat different from our earlier findings. This difference could be due to the high number of biopsies exhibiting PIN in the present study.

Figure 3 Histopathological picture of PIN II without any koilocytosis in this part of the epithelium. No dermal inflammatory infiltrate is present, but some dilated capillaries are seen in the upper dermal papillae.

Not unexpectedly, two of the four biopsies exhibiting PIN III were all associated with the presence of oncogenic HPV types 16/18 while we did not detect HPV DNA in the other two. The presence of PIN III corresponds to the "bowenoid" changes that were originally designated "bowenoid papulosis" alluding to clinically overt papular lesions in young adults of average age in the upper twenties located symmetrically on the outer genitals of both sexes.³³⁻³⁵ Our study underscores the view that the original description of "bowenoid papulosis" merely represents a fraction of HPV-associated severe dysplasia of the outer genital area. As accounted for also by others^{36,37} these cases did not exhibit any colposcopic/penoscopic features distinguishing them from benign lesions. Interestingly, while Gross *et al*³⁴ reported the presence of koilocytosis at a relatively low frequency (5, 1%) in bowenoid papulosis, we found koilocytosis in as much as 75% (3/4; table 3) of lesions exhibiting PIN III.

Balanoposthitis represents a large clinical entity with a wide aetiological spectrum.³⁸ The most common microbiological agent is possibly various candida species, of which *Candida albicans* is most prevalent, but a few cases of *Gardnerella vaginalis*-associated balanoposthitis have also been described.³⁹ However, in many cases the underlying cause remains unknown. While most cases of balanoposthitis represent isolated episodes of short duration, recurrent herpes simplex sometimes induces periodically recurrent balanoposthitis-like complaints. Furthermore, a number of dermatological conditions of unknown aetiology may predispose for more long-lasting symptoms; these include histologically well defined entities such as lichen sclerosus et atrophicus, lichen ruber planus and balanitis circinata parakeratolica.

The present investigation selectively includes men with a broad range of penile inflammatory symptoms including redness, itching, burning, tenderness, dyspareunia and fissuring. In the 18 men with available information the symptoms had recurrently occurred during an average of as much as 11 months (range 0–48). The median duration of symptoms was, on the other hand somewhat shorter, four months. We have been able to show some tentative evidence that might be interpreted as cause-and-effect between GPVI and such long-lasting penile symptoms. We would like to emphasise, however, that our data are still circumstantial for a causative association. Additional prospective HPV DNA studies are required to confirm our hypothesis. Preferably, also RNA analysis should be included for the detection of HPV replication in such lesions.

The symptoms presented by us exhibit a striking resemblance to those described in females as "papillomavirus-vulvovaginitis".⁴⁰ The latter condition is frequently associated with recurrent fissuring of the posterior fourchette of the vulva.⁴¹ In our study recurrent painful fissuring of the fraenum, the coronar sulcus and/or the foreskin was reported in as much as 43% of the men, and

39% of them had experienced periods with dyspareunia.

Accordingly, in congruence with the condition described for the vulva, we suggest the term "papillomavirus-associated balanoposthitis" for the male counterpart. The occurrence of penile oedema and inguinal adenopathy has to our knowledge previously not been associated with GPVI. It is noteworthy that 35% of these men had been treated previously and that penoscopy revealed the presence of tiny residual penile warts in 22% of them. This finding elucidates the empirical fact that current therapy often is suboptimal and that long-term problems may occur in spite of an apparent eradication of clinically visible warts.

The prevalence of HPV-associated penile symptoms is unknown. The present patient material was collected during a rather long period of time (4 years); accordingly, we feel confident that a symptomatology of the severity described here is encountered merely occasionally. Yet, inflammatory reactions associated with GPVI might indeed be more frequent than appreciated so far.^{42,43} Löwhagen *et al*⁴⁴ recently reported that in a series of 88 patients with various macromorphological types of penile GPVI lesions, balanitis was found in as much as 44% of men with macular lesions (7/16). Accordingly, we would like to stress the importance of investigating the potential of an underlying GPVI in the pathogenesis of balanoposthitis, especially in persistent and/or recurrent cases as well as in men with penile lesions of unclear etiology.

In part, an inflammatory reaction may have emanated from an exogenous irritative and/or microbiological stimulus. Although PAS staining did not reveal a fungal infection in any of 15 investigated biopsies, the potential pathogenetic influence from candida cannot be ruled out due to the use of miconazol containing topical remedy prior to biopsy sampling.

The inflammatory response may also represent an HPV induced immunological response.⁴⁵⁻⁵⁰ When virus replication takes place, viral antigens and/or host neoantigens may be expressed on infected keratinocytes and potentially elicit immune regulating cytokines and/or attract immunocompetent lymphocytes. It is well appreciated that spontaneous wart rejection is associated with dermal accumulation of lymphocytes and macrophages that subsequently penetrate into the epidermis. The mere finding of dermal inflammatory cells is common in GPVI lesions⁶ and may possibly represent an early phase of a spontaneous involution process. Thus, some of the symptoms experienced by the men in the present study may possibly represent a favourable prognostic sign, signalling an ongoing initiation of an immunologically mediated rejection mechanism. Other symptoms, such as repetitive epidermal fissuring, on the other hand, may possibly be due to an interaction between exogenous traumas and "come-and-go" fluctuations in virological and biological expression.^{1,51}

Knowledge on the natural course of

acetowhite lesions is quite limited. However, studies on cervical lesions indicate that the majority will clear spontaneously within a period of 5–6 years.¹⁰ For asymptomatic sub-clinical GPVI in men, it seems rational to await spontaneous regression of the lesions as well. However, if the lesions are associated with symptoms such as balanoposthitis, as well as for condylomas and overt bowenoid papulosis, active therapy is required and surgical eradication seems at present to represent the only alternative.

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