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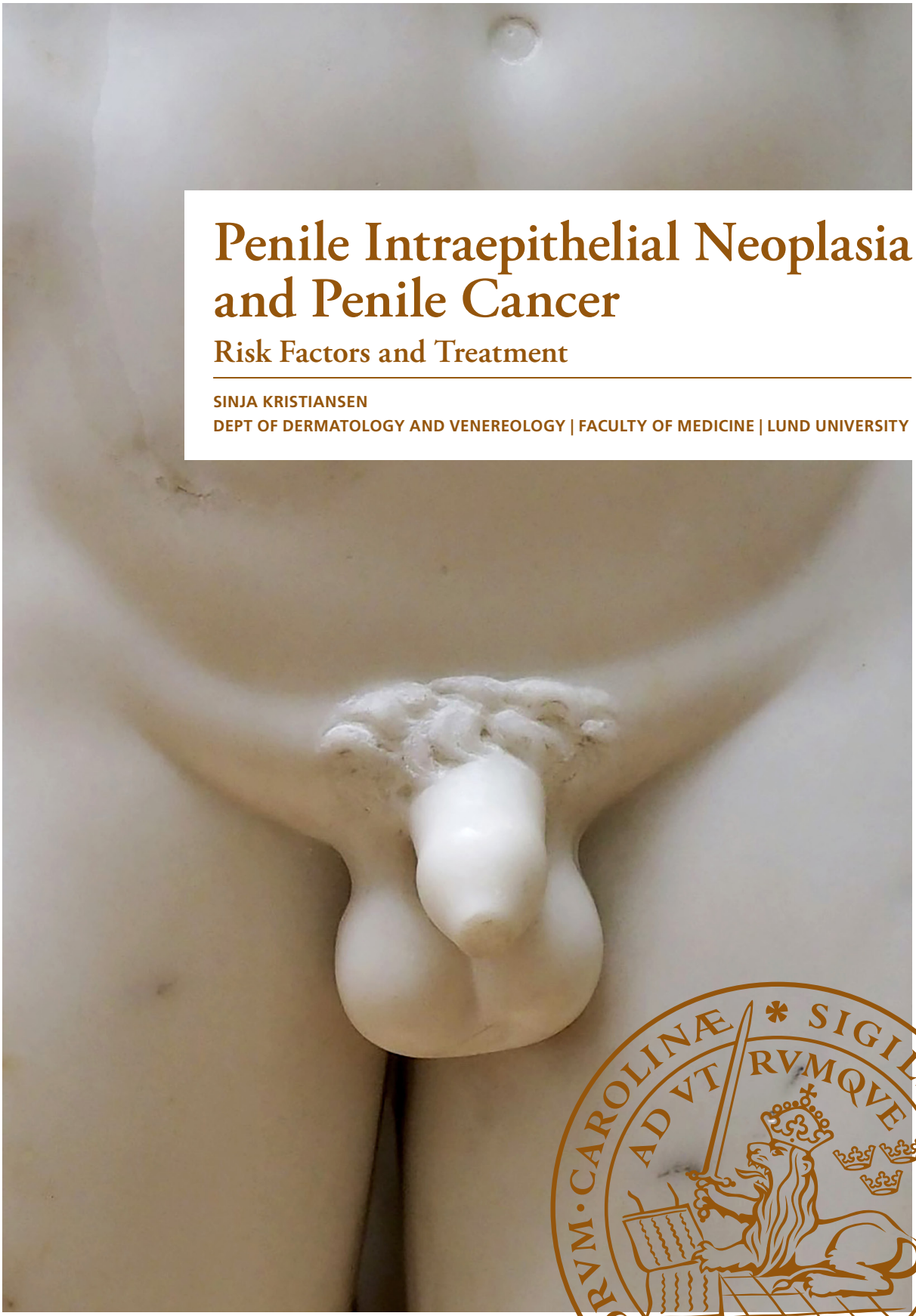

Penile Intraepithelial Neoplasia and Penile Cancer

Risk Factors and Treatment

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Risk Factors and Treatment

Sinja Kristiansen

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Abstract

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The aims of this thesis were to explore the incidence, risk factors and treatment of PeIN; to analyse the prevalence of HPV and skin diseases in circumcised preputium; to investigate the prevalence of HPV in penile cancer compared to age-matched controls; and in HPV16-positive cases, to analyse viral activity.

Risk factors for PeIN, studied in a case-control study of 580 cases and 3436 controls, showed increased odds ratios for lichen sclerosus (LS), lichen planus (LP), genital warts, balanoposthitis, taking immunosuppressive drugs, penile surgical procedures and organ transplantation.

The incidence of PeIN retrieved from the Swedish National Penile Cancer Register over 20 years, revealed an increased standardised incidence rate of 2.37 from 2019 to 2000. A comparison of given treatment for PeIN in the last five years, compared to the first five years of the period studied, showed surgery to be more common than laser treatment and, topical imiquimod and 5-FU to be more common than local destructive methods.

Analysis of symptomatic foreskin (N=351) showed HPV in 17.1% of cases, high-risk (HR) HPV types in 9.1% with HPV16 in only 2.3%. Histologically, LS, LP and lichenoid dermatitis were seen in 73.5% and PeIN in 2%, despite no clinical suspicion of malignancy.

In penile cancers (N=135) HPV was detected in 38.5% of cases and HPV16 was present in 27.4%. Among cases and age-matched controls (N=105) HR HPV types were found in 34.3% (48/135) of tumours and in 4.8% (5/105) of controls ($p<0.001$). Among tumours and controls, HPV16 was present in 27.4% (37/135) and 1% (1/105), respectively ($p< 0.001$). Viral activity (HPV16 mRNA) among HPV16-positive cases was more common in the tumour (86.5%) compared to adjacent to the tumour (21.7%) ($p<0.001$).

In conclusion, this thesis provides knowledge about risk factors, change in incidence, and treatment methods over 20 years for PeIN. For penile cancer, HR HPV types were significantly more common in penile cancer cases than in age-matched controls. The finding of active HPV16 in penile cancer suggests that HPV16 is an oncogenic driver of the disease.

Key words
Penile intrapithelial neoplasia, PeIN, penile cancer, risk factors, HPV, incidence, histopathology

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To Juno and Alve,
because you are and will always be
the best things that have ever happened to me

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Kristiansen S, Svensson Å, Drevin L, Forslund O, Torbrand C, Bjartling C. Risk Factors for Penile Intraepithelial Neoplasia: A Population-based Register Study in Sweden, 2000–2012. Acta Derm Venereol 2019; 99: 315–320.

Paper II

Kristiansen S, Svensson Å, Bjartling C, Forslund O, Torbrand C. Penile intraepithelial neoplasia, penile cancer precursors and human papillomavirus prevalence in symptomatic preputium: a cross-sectional study of 351 circumcised men in Sweden. BJU Int 2021; 127: 428–434.

Paper III

Kristiansen S, Torbrand C, Svensson Å, Forslund O, Bjartling C. Incidence of penile intraepithelial neoplasia and treatment strategies in Sweden 2000 to 2019. BJU Int 2022, Jan 19. Online ahead of print.

Paper IV

Kristiansen S, Bjartling C, Torbrand C, Grelaud D, Lindström M, Svensson Å, Forslund O. Increased prevalence of HPV in fresh tissue from penile cancers compared to non-malignant penile samples. Manuscript 2022.

Preface

As a medical student, I was part of a student organisation called Kärleksakuten. We visited schools and educated children and teenagers about genital anatomy, sex, sexually transmitted infections, and safe sex. I had to wait until the 10th semester of medical school before we reached the course in dermatology and venereology. Finally, I joined the department of sexual health and I still remember the day I decided that this would become my future area of work.

I was supervised by Annika Johnsson, when we had a male patient, about my own age, who had been tested for sexually transmitted diseases three months earlier with negative results. He now came with symptoms of a green-yellow discharge from the urethra. He suggested that he had gonorrhoea, recognising the symptoms since he had had it before. During the examination, we saw several ulcerations on the penis. The patient was not bothered since they gave no symptoms. The clinical diagnosis, verified with a polymerase chain reaction (PCR) test, was primary syphilis with several chancres. Under the microscope, we verified diplococci, confirming the gonorrhoea diagnosis. Upon testing, we found he also had chlamydia and HIV. Of course, he was treated and followed up, and I subsequently became convinced, that this was the area in which I wanted to work and make a difference!

After finishing medical school, I worked for one year in the Department of Urology at Skane University Hospital, focusing on surgery for male genital cancers. I loved the practical and three-dimensional aspects of surgery. After my internship, I was ready to start at the Department of Dermatology and Venereology, with a naïve wish to combine the clinical work with research, and make a difference to the world.

Now, as a specialist in dermatovenereolgy, I think I have the best job in the world. It involves treating so many different types of skin diseases; sometimes it is easy and sometimes you must deliberate and investigate to find the right diagnosis. It is never boring; even if ten patients have psoriasis, they are all different personalities. I love performing surgery on all skin cancer patients because it’s like a manual skill, where you always want to be become better.

Finally, yet importantly, the dimension of research, to pssibly be able to help not only the patient in front of you, but also many patients in the future, is very encouraging. However, I realise that making a difference to the world is a huge task, so I will probably have to settle with the goal of helping just some of the patients with penile diseases.

Abbreviations

AIN	Anal intraepithelial neoplasia
BCC	Basal cell carcinoma
BMI	Body mass index
BP	Bowenoid papulosis
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CO2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
DNA	Deoxyribo nuleic acid
EAU	European Association of Urology
ESP	European standard population
5-FU	Fluorouracil
H&E	Hematoxylin and eosin
HPV	Human papillomavirus
HR	High-risk
HSIL	High-grade squamous intraepithelial lesion
ICD	International classification of diseases
IQR	Interquartile range
IPR	National Inpatient Register
LP	Lichen planus
LR	Low-risk
LS	Lichen sclerosus
LSIL	Low-grade squamous intraepithelial lesion
MM	Malignant melanoma

mRNA	messenger Ribo Nucleic Acid
MSM	Men having sex with men
Nd:YAG	Neodymium:yttrium-aluminium-garnet
NMSC	Non-melanoma skin cancer
NPECR	National Penile Cancer Register
OPR	National Outpatient Register
OR	Odds ratio
PCR	Polymerase chain reaction
PDT	Photodynamic therapy
PeIN	Penile intraepithelial neoplasia
PenCBaSe	Penile Cancer Base Sweden
PUVA	Psoralen and UVA
RCT	Randomised controlled trial
RPD	National Register of Prescribed Drugs
RR	Relative risk
SCC	Squamous cell carcinoma
SCR	Swedish Cancer Register
SIR	Standardised incidence rate
TGR	Total glans resurfacing
UVB	Ultraviolet B
VIN	Vulvar intraepithelial neoplasia
WLE	Wide local excision
WHO	World Health Organization

Abstract

Penile cancer and its precursor, penile intraepithelial neoplasia (PeIN), are rare malignancies. Data on risk factors, incidence, and treatment of PeIN is scarce. The prevalence of human papillomavirus (HPV) varies substantially between studies of penile cancer.

The aims of this thesis were to explore the incidence, risk factors and treatment of PeIN; to analyse the prevalence of HPV and skin diseases in circumcised preputium; to investigate the prevalence of HPV in penile cancer compared to age-matched controls; and in HPV16-positive cases, to analyse viral activity.

Risk factors for PeIN, studied in a case-control study of 580 cases and 3436 controls, showed increased odds ratios for lichen sclerosus (LS), lichen planus (LP), genital warts, balanoposthitis, taking immunosuppressive drugs, penile surgical procedures and organ transplantation.

The incidence of PeIN retrieved from the Swedish National Penile Cancer Register over 20 years, revealed an increased standardised incidence rate of 2.37 from 2019 to 2000. A comparison of given treatment for PeIN in the last five years, compared to the first five years of the period studied, showed surgery to be more common than laser treatment and, topical imiquimod and 5-FU to be more common than local destructive methods.

Analysis of symptomatic foreskin (N=351) showed HPV in 17.1% of cases, high-risk (HR) HPV types in 9.1% with HPV16 in only 2.3%. Histologically, LS, LP and lichenoid dermatitis were seen in 73.5% and PeIN in 2%, despite no clinical suspicion of malignancy.

In penile cancers (N=135) HPV was detected in 38.5% of cases and HPV16 was present in 27.4%. Among cases and age-matched controls (N=105) HR HPV types were found in 34.3% (48/135) of tumours and in 4.8% (5/105) of controls ($p<0.001$). Among tumours and controls, HPV16 was present in 27.4% (37/135) and 1% (1/105), respectively ($p< 0.001$). Viral activity (HPV16 mRNA) among HPV16-positive cases was more common in the tumour (86.5%) compared to adjacent to the tumour (21.7%) ($p<0.001$).

In conclusion, this thesis provides knowledge about risk factors, change in incidence, and treatment methods over 20 years for PeIN. For penile cancer, HR HPV types were significantly more common in penile cancer cases than in age-

matched controls. The finding of active HPV16 in penile cancer suggests that HPV16 is an oncogenic driver of the disease.

Populärvetenskaplig sammanfattning på svenska

Peniscancer är en ovanlig cancerform. I Sverige är det ca 150 män som insjuknar varje år. Den vanligaste typen av peniscancer utgörs av skivepitelcancer. Ytlig skivepitelcancer på penis kallas penil intraepitelial neoplasi (PeIN).

Det finns två huvudsakliga risker som är kopplade till peniscancer humant papillomvirus (HPV) och inflammatoriska hudsjukdomar så som lichen sclerosus (LS) och lichen planus (LP).

Behandlingen för peniscancer är kirurgisk excision. För PeIN finns både kirurgiska och andra behandlingsalternativ som t.ex. kräm-, frys- och ljusbehandling.

Prognosen vid peniscancer är god. Den totala 5-årsöverlevnaden är 82% och vid PeIN samt lokal peniscancer är den över 90%.

Forskningen kring PeIN och dess konsekvenser är begränsad. Målet med denna avhandling var att ta reda på vilka riskfaktorer som finns för PeIN? Ökar antalet fall av PeIN över tid? Vilken behandling får patienter med PeIN? Förekommer PeIN och inflammatoriska hudsjukdomar i ökad utsträckning i förhuden hos patienter med symtom från förhuden? Målsättningen var också att ta reda på förekomsten av HPV vid peniscancer och undersöka om viruset är aktivt i tumören jämfört med hos kontroller utan cancer.

Beträffande riskfaktorer för PeIN, studerades 580 patienter med PeIN ur svenska peniscancerregistret. Dessa patienter jämfördes med 3436 kontroller utan PeIN, matchade avseende ålder och boendelän. Ökad risk för PeIN sågs hos män med inflammation på förhud och/eller ollon, genitala vårtor, LS, LP, samt män som medicinerade med immunsänkande läkemedel eller tidigare hade genomgått kirurgiska ingrepp på penis.

Alla PeIN-fall i det nationella peniscancerregistret från år 2000 till 2019 undersöktes gällande antal nyinsjuknade i PeIN samt vilken behandling de fått i relation till tumörstorlek, tumörlokalisation, ålder och komplikationer. Resultaten visade att PeIN ökar i Sverige. Det är 2 ggr vanligare att få en PeIN-diagnos 2019 jämfört med år 2000, även när det justeras för att befolkningen har ökat. När olika typer av behandling som givits över tid utforskades, visade det sig att de 5 sista åren erhåller

patienter oftare kirurgisk excision jämfört med laserkirurgi och krämbehandling istället för lokalt destruerande metoder.

I ett delarbete inkluderades 351 män över 18 år som genomgick circumcision (omskärelse) pga. symtom från förhuden. Under operationen togs en ca 5 mm stor förhudsbit och skickades till Labmedicin i Lund för HPV analys. Resten av förhuden skickades för histopatologisk bedömning. HPV påvisades hos 17.1%, varav 9% hade högrisk HPV, men endast 2.3% hade HPV16 (som är den vanligast förkommande HPV-typen vid peniscancer). I 2% av de 351 fallen återfanns PeIN i förhuden, trots att doktorn inte hade haft någon klinisk misstanke om detta före operationen. Närmare 60% hade LS, 5.7% hade LP och 9.1% hade en lichenoid dermatit. Endast 13% hade en histopatologiskt normal förhud.

I det sista delarbetet undersöktes förekomst av HPV hos 135 peniscancerpatienter och 105 åldersmatchade kontroller. Vid förekomst av HPV16 analyserades virusets aktivitet. Förekomsten av HPV hos peniscancerfallen var 38.5% att jämföra med 11.4% hos kontrollerna. HPV16 förekom hos 27.4% hos peniscancerpatienterna och 1% av kontrollerna. Aktivitet hos HPV16 påvisades oftare i tumören jämfört med 10 mm vid sidan av tumören, vilket visar på virusets betydelse för cancerutvecklingen.

Sammanfattningsvis så har de ingående delarbetena i avhandlingen visat på liknande riskfaktorer för ytlig peniscancer som för invasiv peniscancer, dvs inflammation på förhud och/eller ollon, genitala vårtor, LS, LP, att stå på immunsänkande läkemedel samt att tidigare ha genomgått peniskirurgi. Detta understryker vikten av att behandla och följa upp hudsjukdomar på penis. Det pekar också på betydelsen av att informera om och rekommendera HPV vaccin till alla barn, vilket sedan hösten 2020 ingår i skolvaccinationsprogrammet oavsett kön.

Förekomsten av PeIN utan klinisk misstanke hos patienter med symtom från förhuden samt den höga förekomsten av inflammatoriska, behandlingsbara hudsjukdomar visar på vikten av att alltid skicka bortopererad förhud för histopatologisk bedömning. När inflammatorisk hudsjukdom påvisas, som potentiellt genom ärrbildning kan ge ett trångt urinrör och i vissa fall även ytlig cancer, ska patienten informeras och vid kvarvarande sjukdom behandlas och följas upp av läkare med kunskap inom området genitala hudsjukdomar.

En ökande förekomst av PeIN, poängterar att trots att det är en ovanlig sjukdom, så behöver läkare under utbildning och läkare i primärvården känna till diagnosen och veta när de skall remittera vidare. Urologer och dermatovenereologer behöver också utbildas kring utredning och behandling av PeIN. Då det finns många olika behandlingar för PeIN, men inga jämförande studier kring vilken behandling som har bäst utläkning och ger minst risk för återfall, behövs mer forskning och ökad kunskap, vilket bland annat fås genom den multidisciplinära teambedömning som tillhandahålls för alla fall av peniscancer och PeIN i Sverige sedan 2013.

Fynd av HPV och virusaktivitet i peniscancertumören pekar på HPVs roll i cancerutvecklingen och styrker rekommendationen att även pojkar ska vaccineras, vilket framöver kan leda till immunitet i samhället och att vi därigenom kan begränsa förekomst av både peniscancer, vulvacancer, analcancer och livmoderhalscancer.

Introduction

Overall introduction

Penile cancer is a rare malignancy. In Sweden there are approximately 150 new cases every year (1). Usually, men are aged 60-70 when diagnosed with penile cancer.

The majority of penile cancers are histologically defined as squamous cell carcinomas (SCC) where the malignant transformation starts with dysplasia in the epithelium. Full thickness dysplasia is called penile intraepithelial neoplasia (PeIN) and is an SCC in situ.

The two major pathways to malignancy in squamous cells on the penis are associated with human papillomavirus (HPV), and inflammatory skin diseases such as lichen sclerosus (LS) and lichen planus (LP). A variety of treatment methods within the surgical and topical field are available for PeIN lesions, but for invasive SCC the tumour must be excised.

The overall five-year relative survival rate in Sweden is 82%, with a very good prognosis in PeIN (five-year survival rate 97%) and in localised invasive penile cancer (pT1) (five-year survival rate 90%) (1).

Epidemiology of penile cancer

Penile cancer is a rare type of cancer with a global age-standardised incidence of 0.8 per 100 000 person-years (2). In Sweden, the age-adjusted incidence is 2.1/100 000 person-years (1). In Europe the age-standardised incidence is 0.45 – 1.7/100 000 person-years (3) and in the USA it is 0.58/100 000 person-years (4). Brazil has the highest reported incidence in the world with an age-standardised incidence rate of 6.15/100 000 person-years over a five-year period (5) (Table 1).

The age-standardised incidence rate of penile cancer in Uganda has also been high with 3.3/100 000 person-years, but recently data has shown a decline in incidence to 1.2/100 000 person-years (6).

A declining incidence was also seen both among black and white men in the USA between 1973 and 2003 (7). In contrast, an increasing incidence was seen in the

Netherlands, the UK and Germany (8-10). In the Netherlands, the increase was from 1.4/100 000 person-years in 1989 to 1.5 in 2006 (8), compared to an age-standardised increase in UK from 1.1/100 000 person-years 1979 to 1.3 in 2009 (9) and an age-standardised incidence in Germany of 1.2/100 000 person-years in 1961 to 1.8/100 000 in 2012 (10). In Norway, a moderate increase of penile cancer incidence was seen between 1956 and 2015 (11).

In Denmark, the incidence of penile cancer remained stable between 1978 and 2010 (12). Also in Sweden, the incidence of invasive penile cancer was stable between 2000 and 2012 (1). In France, Australia and Canada a stable incidence of invasive penile cancer was shown from 1989 to 2011, 1977 to 2013 and 1992 to 2010 respectively (13-15).

Table 1.
Incidence of penile cancer/100 000 person-years in different countries.

Country	Period studied	Incidence/100 000	Trend
Sweden	2000-2012	2.1	Stable
Denmark	1978-2010	1.05	Stable
France	1989-2011	0.59	Stable
Canada	1992-2010	0.61	Stable
Australia	1977-2013	0.46	Stable
Norway	1956-2015	0.91	Increasing
The Netherlands	1989-2006	1.5	Increasing
Germany	1961-2012	1.8	Increasing
United Kingdom	1979-2009	1.3	Increasing
The United States	1973-2003	0.58	Declining
Brazil	2004-2014	6.15	Declining
Uganda	1991-2010	1.2	Declining

Incidence of PeIN

PeIN is a premalignant precursor lesion of invasive penile cancer, a SCC in situ, where the squamous epithelium shows dysplastic changes with an intact basement membrane (16).

In about 150 penile cancer cases diagnosed per year in Sweden, approximately 30% are diagnosed with PeIN (1).

The mean incidence of PeIN in the Netherlands is 0.47/100 000 person-years. The relatively low number is deemed to be due to potential underestimation, as premalignant lesions may not be recognised by the patient or the clinician (17). In the same study an increasing incidence of PeIN was shown with an overall increase in incidence rate of PeIN from 0.4/100 000 person-years in 1998 to 0.6/100 000 person-years in 2007 (17).

Data on the incidence of PeIN from the National Cancer Institute`s Surveillance, Epidemiology and End Results programme in the USA showed that PeIN constituted 37% (595/1605) of penile cancer cases, with a tendency to increase between 1973 and 1998 (18).

A Danish study by Baldur-Felskov et al. investigated the National Pathology Data Bank from 1998 to 2008 and found 285 cases of PeIN 2 and 3 (according to the former classification of PeIN), with a calculated age-standardised incidence of 0.5/100 000 men-years in 1998-1999 compared to 0.9/100 000 men-years in 2006-2008 (19). As pointed out by Frish et al. when commenting on the article by Baldur-Felskov et al., it should be noted that any recurrence of PeIN 2 and 3 occurring more than 24 months after the initial PeIN 2/3 was counted as a new PeIN, maybe explaining some of the increased incidence (20).

A recent study from Denmark investigating the incidence of PeIN between 1997 and 2018 shows an increase in age-standardised incidence from 0.87 (95% CI 0.65-1.16) per 100 000 person-years in 1997-1998 to 1.84 (95% CI 1.55-2.20) in 2017-2018 (21).

Definition of penile cancer and PeIN

Histological classification of invasive penile cancer

Penile cancer is histologically diagnosed as SCC in 94-99% of cases (1, 8, 22). Other non-squamous malignancies of the penis constitute, in descending order of frequency: Kaposi sarcoma, malignant melanoma (MM), basal cell carcinoma (BCC) and extra mammary Paget's disease (23).

The classification of squamous cell carcinomas of the penis was changed in 2016 by the World Health Organization (WHO) and was a result of a gradual historical evolution of the nomenclature and clinico-pathological understanding of SCC. Scientific evidence resulted in the hypothesis of the bimodal pathogenesis of penile cancer into HPV-related and non-HPV-related. The histological morphological subtypes of penile squamous cell carcinomas associated with HPV are basaloid, papillary-basaloid, warty, warty-basaloid, clear cell and lymphoepithelioma-like carcinomas (Table 2).

Table 2
Classification of squamous cell carcinoma of the penis by WHO

Non-HPV-related penile SCC	HPV-related penile SCC	Other
SCC	**Basaloid carcinoma**	**Unclassified carcinoma**
Usual carcinoma	**Papillary-basaloid carcinoma**	
Pseudohyperplastic carcinoma	**Warty carcinoma**	
Pseudoglandular carcinoma	**Warty-basaloid carcinoma**	
Verrucous carcinoma	**Clear cell carcinoma**	
Pure verrucous carcinoma	**Lymphoepitelioma-like carcinoma**	
Carcinoma cuniculatum		
Papillary carcinoma, NOS*		
Adenosquamous carcinoma		
Sarcomatoid squamous carcinoma		
Mixed carcinoma		

*Not otherwise specified

The non-HPV-related histological subtypes of squamous penile carcinomas are called: usual type, pseudohyperplastic type, pseudoglandular type, verrucous types, papillary, adenosquamous, sarcomatoid and mixed carcinomas. For histopathological pictures of different subtypes of penile squamous cell carcinomas see figure 1.

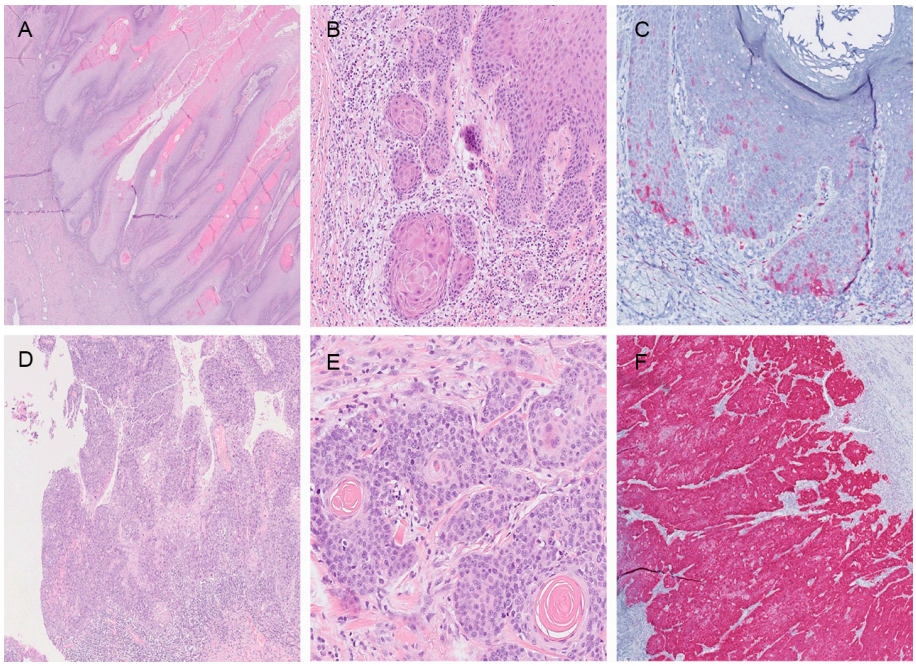

Figure 1
Different histological subtypes of squamous cell carcinoma of the penis. **A** (top left). Verrucous carcinoma. Note exophytic growth pattern and pushing borders. **B** (top middle). Invasive squamous cell carcinoma, usual type. Note basal and parabasal atypia with retained keratinisation and a mild stromal response. **C** (top right). P16-immunohistochemical staining, in rare instances, showed a non-specific focal and cytoplasmatic positivity in cancer of usual types and associated differentiated PeIN. **D** (bottom left). Warty carcinoma. Exophytic growth pattern with complex fused papillae and poorly defined borders. **E** (bottom middle). Invasive basaloid carcinoma. Note abrupt keratinisation and slight stromal response. **F** (bottom right). Typical positive reaction for P16 in basaloid carcinoma.

Cubilla et al. have shown that the usual type of penile SCC is the most prevalent subtype represented in 44% of penile cancer cases, followed by mixed types (tumours with > one histological pattern) seen in 21%. Sarcomatoid, basaloid and warty subtypes are represented in 7% each, followed by warty-basaloid in 4%, pseudohyperplastic in 3% and verrucous, papillary and other types in 2% each (24).

Histological classification of PeIN

PeIN, the penile SCC in situ and precursor lesion to invasive penile SCC, is graded according to the WHO classification into HPV-related PeIN based on HPV, named *undifferentiated* PeIN and non-HPV-related PeIN, originating from inflammatory skin diseases such as LS and LP, named *differentiated* PeIN (16).

PeIN is morphologically divided into four subgroups with *differentiated* PeIN (histology seen in figure 2) being the most predominant, and seen in 68% of PeIN

cases, followed by warty-basaloid in 14%, basaloid (histology seen in figure 3) in 11% and warty in 7% (histology seen in figure 4) (25).

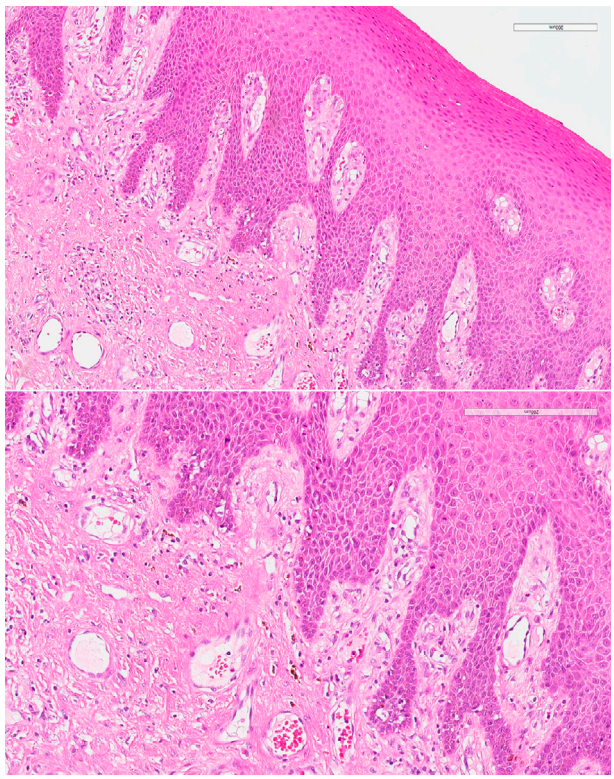

Figure 2
Histology of *differentiated* PeIN. Close-up below.

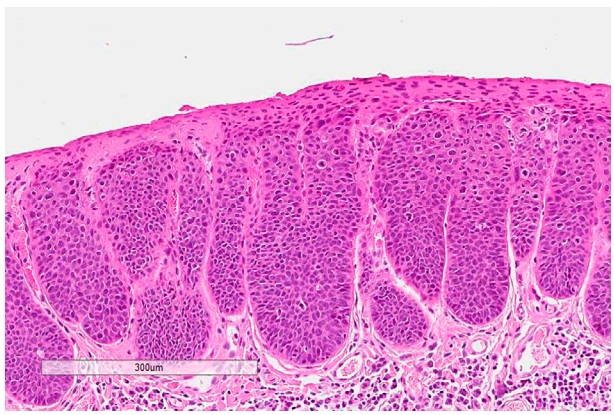

Figure 3
Histology of *undifferentiated* PeIN, basaloid subtype.

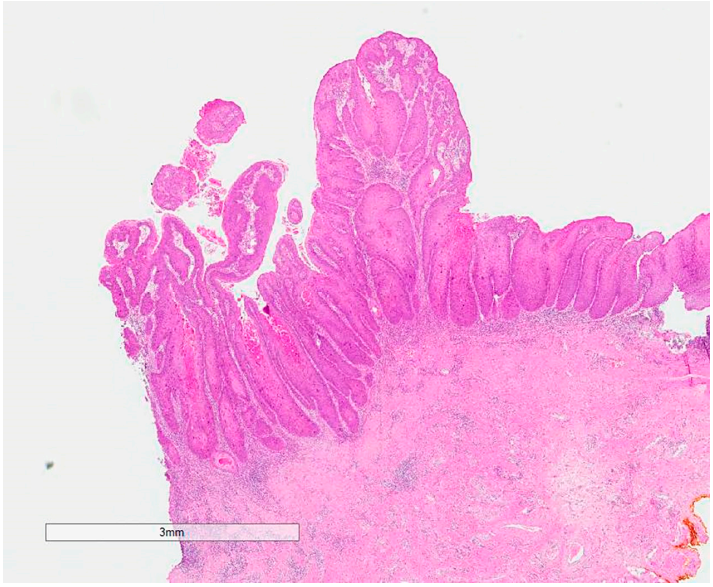

Figure 4
Histology of *undifferentiated* PeIN, warty subtype.

The new classification does not consider the historically used grading into PeIN I, II and III, reflecting different stages of involvement of the dysplasia of the epithelium. One reason for the changed classification of PeIN with the abolishment of separation into PeIN I, II and III is that the assessment of degree of dysplasia in the epithelium varies, even among experienced pathologists (26, 27).

This change is in alignment with the changed classification of vulvar intraepithelial neoplasia (VIN), where non-HPV-related VIN is called *differentiated* VIN (dVIN) and HPV-related VIN is divided into the high-grade squamous intraepithelial lesion (HSIL) and the low-grade squamous intraepithelial lesion (LSIL), the latter consisting of genital warts or effects of genital warts (28).

Also in cervical intraepithelial neoplasia (CIN) the classification changed in 2014 into LSIL and HSIL, the latter including CIN II and CIN III, still divided according to the old classification into CIN II and CIN III, due to clinical relevance in women younger than 27 years (29).

In anal intraepithelial neoplasia (AIN) the classification has also changed into LSIL and HSIL, since the reproducibility of grading the intraepithelial neoplasia into AIN I, II and III is low (27, 30). In intermediary lesions (formerly called AIN II), staining for P16 is used to help guide pathologists with the diagnosis, but still a study by Liu et al. showed that although P16 increased inter-observer agreement among experienced pathologists, considerable disagreement remained regarding intermediate lesions (31).

TNM classification

TNM (Classification of Malignant Tumours) is a globally recognised standard for classifying the extent of spread of cancer. The T category describes the primary tumour regarding site and size, the N category describes the regional lymph node involvement, and the M category denotes whether distant metastases are present. The latest classification is the TNM 8th where PeIN is named Tis, with T standing for tumour and *is* standing for carcinoma in situ (32, 33).

International Classification of Diseases

The International Classification of Diseases (ICD) is a globally used classification system of diseases and procedures allowing for international comparability in collecting, processing, classification and presentation of morbidity and mortality statistics.

The version currently in use is called ICD-10, but a revised version called ICD-11 has been launched in English and a translation into Swedish is planned to be finished in 2024. According to ICD-10, malignant neoplasms of the penis are coded as C60. PeIN is called carcinoma in situ of the penis and is coded as D074 (34).

Clinical classification of PeIN

The clinical classification of PeIN has changed over time. A PeIN in keratinised skin was historically called Morbus Bowen in analogy with SCC in situ elsewhere on the skin, but later changed name several times (35). John Templeton Bowen (1857-1940) was an American dermatologist, giving his name to Bowen's disease in 1912 (36).

A distinct and well-demarcated plaque that can be skin-coloured, red or brown characterises PeIN on keratinised skin (Figure 5). On the glans or the inner prepuce, where the skin consists of a mucous membrane, PeIN is characterised by a red, moist plaque (Figure 6 and 7), formerly often called Erythroplasia of Queyrat (35, 37, 38), Erythroplasia meaning red and Queyrat, after the French dermatologist and syphilologist Vincent Jules Louis Queyrat (1856-1933), named in 1911 (39).

The most recent classification is into *undifferentiated* PeIN, when derived from HPV and *differentiated* PeIN when arising in the background of inflammatory skin diseases such as LS and LP, regardless of the clinical appearance (16).

Symptoms of PeIN can vary among no symptoms, irritation, dryness, itching, pain, bleeding, fissures and phimosis (40, 41, 42).

Differential diagnoses of PeIN are genital skin diseases such as LS, LP, psoriasis, eczema, balanopostithis and plasma cell balanitis. Differential diagnoses can also

be sexually transmitted diseases such as syphilis, herpes, genital warts or Bowenoid papulosis (BP) (41-43). The latter is a clinical entity, despite being histologically identical to PeIN, characterised by multiple brown-red lesions that are often HPV-positive (Figure 8).

Treatment options in BP are similar to treatment of genital warts, and studies have shown < 1% risk of BP becoming an invasive penile cancer (37, 44-46).

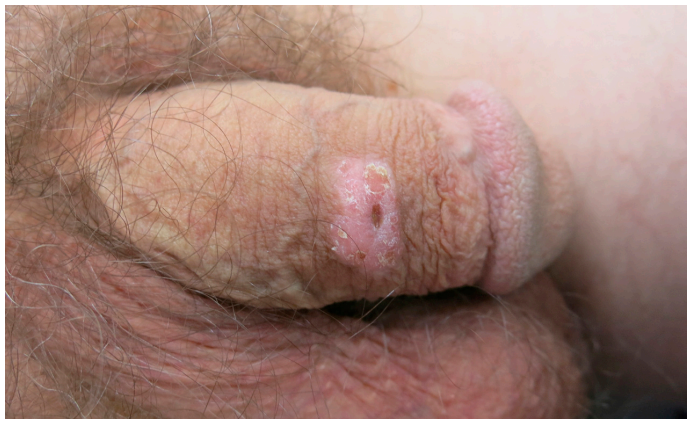

Figure 5
Clinical picture of PeIN on keratinised skin. Published with permission of the patient.

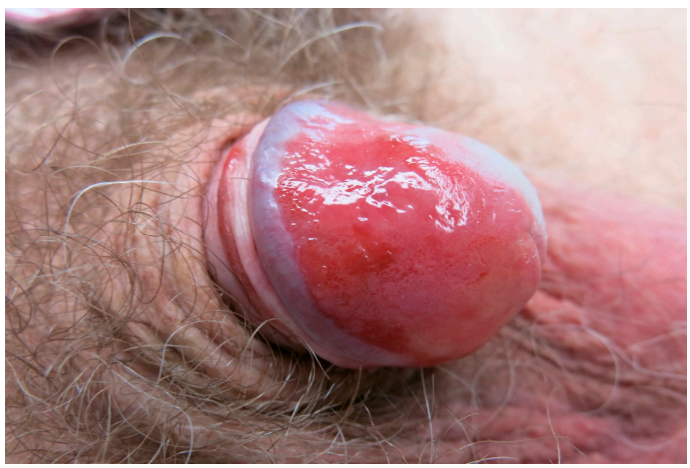

Figure 6
PeIN on a mucous membrane. Published with permission of the patient.

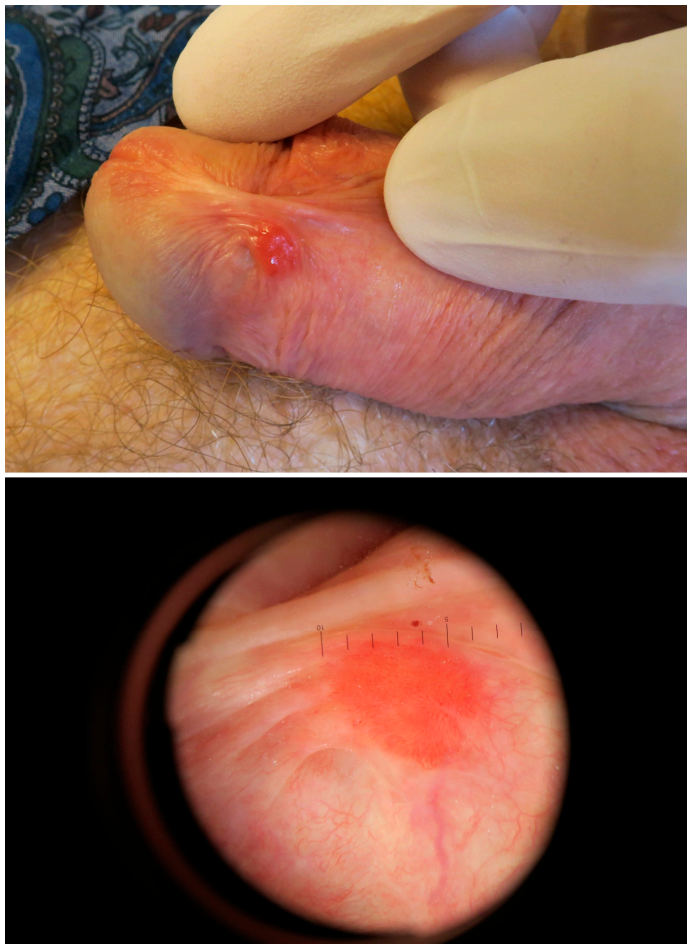

Figure 7
PeIN next to frenulum. Dermoscopy picture of the PeIN lesion below. Published with permission of the patient.

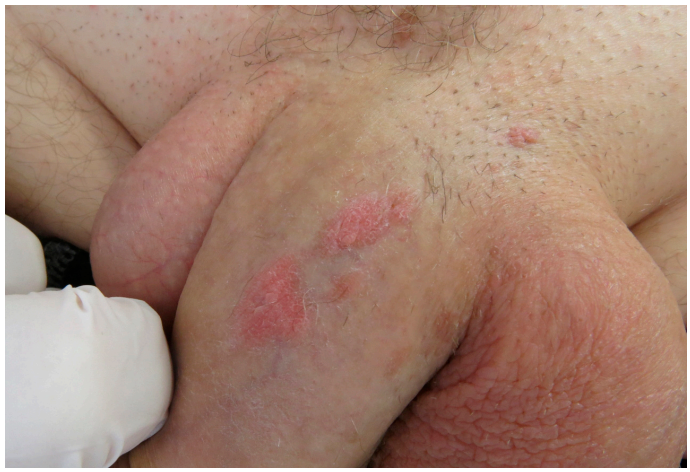

Figure 8
Bowenoid papulosis, a clinical diagnosis, despite having a histological appearance as a PeIN. Published with permission of the patient.

The anatomical locations of the PeIN are described by Hoekstra et al. to be most often on the prepuce (45%), followed by the glans (38%) and seldom on the penile shaft (3%) (17). Contrary to this, Chipollini et al. found PeIN located on the glans to be more common than on the prepuce, 44.9% of PeIN lesions compared to 21.5%. Consistent with Hoekstra et al. they also found location on the penile shaft to be the least prevalent, described as occuring in 4.9% of PeIN cases (47). For different locations of PeIN see figure 9.

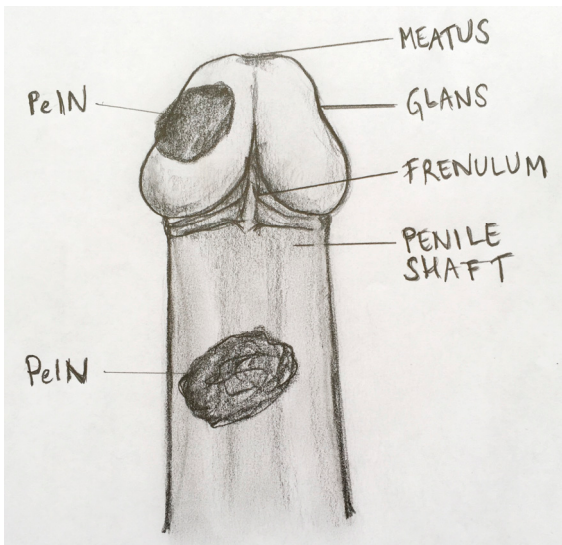

Figure 9
Different anatomical locations of PeIN.

Aetiology of penile cancer and PeIN

The aetiology of penile cancer points to two main pathways of carcinogenesis, one pathway derived from HPV and the other from inflammatory skin diseases such as LS and LP (16, 48).

Human papillomavirus

Studies of HPV in invasive penile cancer have shown HPV to be present in approximately 50% of cases but with a wide range between 24% and 89% (49-52). The wide range of HPV prevalence is argued to be because of different subtypes of penile SCC and different methods used for HPV analysis.

According to WHO, different HPV types are classified into low-risk (LR) types and high-risk (HR) types, depending on their oncogenic potential. The HR types are HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. HPV30 are classified as intermediary HR (53). The most common HPV types in invasive penile cancer are in descending order: HPV16, HPV6/11, HPV18, HPV31, HPV45 and HPV33 (49, 50, 52, 53).

A recent meta-analysis assessing the strength of the link between HPV and penile cancer showed a 4.5-fold increased pooled relative risk for invasive penile cancer in cases with seropositive HPV infection. This is much lower than associations seen for HPV in cervical cancer (54).

The most common method of analysis of HPV in penile cancer is to use paraffin-embedded tissue, but Iftner and Villa argued in 2003 that this method is inferior to HPV analysis of fresh tissue. They also claim that it is important to cut blank blocks between samples to avoid contamination of HPV between samples (55). The importance of cutting blank blocks is further described by Faust et al. who reported HPV positivity in 8% of blank blocks in cervical cancer (56).

Only a few studies have used fresh penile cancer tissue for HPV analysis (51, 57, 58). HPV was found in 89% (49/55) of penile cancer cases by Martin et al. who reported that the high amount was due to analysing fresh tissue (51). In a study comparing HPV analysis in fresh tissue and paraffin-embedded tissue from the same penile tumour, HPV was found in 56% of cases in fresh tissue and in 26% in paraffin-embedded tissue (57). In a recent study by Huang et al., HPV prevalence was 38% (41/108) of frozen samples of penile cancer, which were managed carefully to prevent cross-contamination (59).

The HPV prevalence is highest in basaloid and warty-basaloid penile cancer subtypes, seen in pooled data in 84.0% of cases and 75.7% respectively (52). As a surrogate marker for transcriptionally active HR HPV the immunohistochemical staining P16 can be used for histopathological diagnosis of penile cancer specimens (48).

Analysis of viral load and mRNA expression of HPV is seldom studied in penile cancer but is well studied in cervical cancer (60, 61). Viral DNA, RNA, and antibodies to HPV L1, E6 and/or E7 proteins were studied by Heideman et al. in 83 penile cancers, demonstrating E6 transcriptional activity in 75% (18/24) of HPV16-positive SCCs. They also showed a median viral load of 72 genome copies per cell (range 8.5 to 2667) in HPV16 mRNA-positive cancers (62).

Huang et al. investigated the integration pattern of HPV in penile cancer through high-throughput viral integration detection and found integrated HPV in 92.1% of cases (35/38). Unlike in cervical cancer, the HPV E2 gene was not prone to involvement in the integration (59).

The pooled HPV prevalence in PeIN has been shown to be 80%, with the most common HPV types being the same as in invasive penile cancer (52).

HPV is said to be the most common sexually transmitted infection in the world. Most commonly, HPV infections are asymptomatic, and when symptomatic, genital warts are the most common clinical picture. In genital warts, HPV6 and 11 are common HPV types (53).

The penile HPV prevalence in asymptomatic males has been studied mostly by swab samples and has been shown to be between 16 and 50% among asymptomatic males, partly depending on age (63-66). Data from the USA, Mexico, and Brazil in the HPV in Men (HIM) study showed in a subgroup analysis of 1159 men, that men have a stable risk of acquiring HPV throughout life, contrary to women where the risk of HPV decreases with age.

Acquisition of oncogenic HPV was significantly increased when men had > 10 lifetime female sexual partners compared to not more than one, and for men with at least three anal male-sex partners in the last three months. In the HIM study, 11% of subjects (122/1159) defined themselves as men having sex with men (MSM) (65).

One possible explanation for the decreasing proportion of HPV in older women is the anatomical changes with successive withdrawal of the transformation zone. HPV was found in 50% of men and the oncogenic HPV types with the highest incidence were HPV16, 51, 52 and 59 (65).

Though HPV is sexually transmitted, a study by de Brujin et al. has shown that female partners of patients with penile cancer do not have more premalignant cervical lesions than the general population (67). However, a systematic review suggests the possibility of a small, increased risk in HPV-related cancers among spouses of patients with HPV-related cancer (68).

HPV vaccination

Among HPV vaccines the bi-valent vaccine covering HPV16 and 18 was first approved, then the quadrivalent vaccine covering also HPV6 and 11 was released.

In 2015, the nine-valent vaccine covering HPV6, 11, 16, 18, 31, 33, 45, 52 and 58 was approved and proved safe and effective in both women and men (69, 70).

Many countries have a vaccination programme for girls. In Sweden it started in 2012 as part of the school vaccination program offered to girls at 12 years of age, and includes catch-up vaccination of women up to age 19 (71). The vaccination coverage in girls in Sweden is high, 87.6% for girls born in 2008 for the first dose. Today only the nano-valent vaccine is provided (72).

Since 2020, all children in 5-6th grade in Sweden have been included in the school HPV vaccination programme (72, 73). Including boys in the school-based vaccination programme as late as in 2020 can be compared with Australia including boys already in 2013 (74).

A recently published meta-analysis of the effect of HPV vaccination programmes for girls, including 60 million individuals and up to eight years of post-vaccination follow-up, showed compelling evidence of significantly decreased HPV infections, genital warts and CIN II in girls and women and a significantly decreased number of genital warts in boys and young men due to herd immunity (75). In 2018, WHO called for action to eliminate cervical cancer, but this would need a large scale-up of HPV vaccination with a coverage of 80-100%, as well as screening and treatment of pre-cancer (76).

Lichen sclerosus

LS is an inflammatory skin disease of unknown origin occurring in both men and women. In a study by Velazquez et al. LS was present at the diagnosis of invasive penile cancer in 33% (68/207) of cases (77). In another study by Philippou et al. investigating LS in invasive penile cancer, LS was found in 23.3% of cases (52/223) (78). Perceau et al. reported 44% (8/18) of cases had penile SCC with clinical and histologically associated LS (79). The highest frequency of LS in penile cancer is 55% (11/20), reported by Powell et al. (80).

LS in PeIN is found in 29% (10/34) of pathology reports in consecutive cases reported from one centre by Pietrzak et al. (81). Chaux et al. studied LS in 121 patients with PeIN lesions of which 78.5% (95/121) had concomitant invasive penile cancer. LS was present in 35% of cases (42/121), with 38 of the cases in differentiated PeIN with SCC (30 cases) and without SCC (eight cases) (25). A recently published study by Ashley et al. found co-existent LS in 24.1% of 108 PeIN cases (82).

The risk of developing PeIN and penile cancer in pre-existing LS is said to be between 2% and 13.6% (83, 84). Kravvas studied 301 cases of LS and found 13.6% (41/301) with concomitant LS and PeIN (84). Barbagli et al. re-evaluated the histopathology of 130 men with LS and found invasive penile cancer in 5.4% (7/130) and PeIN in 2.3% (3/130) (85).

No data on treating LS in men to prevent the development of penile cancer exist, but a longitudinal prospective cohort study of 507 women with LS shows that preventive long-term treatment improves genital function, relieves symptoms, reduces the development or progression of scarring, and eliminates the risk of cancer development (86). Since morphologically it is the same disease in men and women it would be likely that well treated LS in men could prevent development into penile cancer, but this has yet to be proved by research.

Lichen planus

LP is another inflammatory skin disease affecting the genital area. Isolated case reports of LP as a risk factor for penile cancer are found and one study by Mannweiler et al. where pathology reports of 35 HPV negative penile cancer cases were studied, showed nine cases of concomitant LP and invasive penile cancer (87). LP has been shown to increase the risk of vulvar cancer with a standardised incidence rate (SIR) of 1.99 (95% CI 1.18-3.13) (88).

LP as a risk factor for PeIN is not well-studied, but case reports exist (89).

Balanoposthitis

Hellberg et al have shown an increased relative risk (RR) for invasive penile cancer in patients with balanoposthitis, RR 9.5 (95% CI 5.2-17.2), declining to 5.22 when adjusted for phimosis (90).

The association between balanoposthitis and PeIN has not been well studied, though Maden et al. studied the risk of PeIN in balanitis and found no association (91).

Phimosis

Phimosis is a prepuce that cannot be retracted over the glans due to narrowing of the preputial orifice and/or adhesion between the glans and the prepuce. Most boys are born with a physiological phimosis, called a primary phimosis, which resolves spontaneously over the years. If a phimosis is persistent or acquired in adult age, it is called secondary phimosis.

By the age of ≥ 18 years, the overall prevalence of phimosis varies between 0.5% and 13% (92). Secondary phimosis is often a consequence of an underlying disease such as an inflammatory skin disease, for example balanoposthitis, LS or LP (93, 94). Acquired phimosis could also be due to sexually transmitted infections, diabetes, obesity, PeIN or invasive penile cancer (90).

Daling et al. have shown an increased OR of 11.4 (95% CI 5.0-25.9) for invasive penile cancer in patients with a history of phimosis and an OR of 3.8 (95% CI 1.4-

10.1) for PeIN (95). In a study from Brazil in a high-endemic area of penile cancer, Oertell et al. investigated 100 consecutive symptomatic circumcised specimens and found PeIN in 30% (30/100), LS in 53% (53/100) and invasive penile cancer in 11% (11/100) (96).

Circumcision

Circumcision is defined as a surgical excision of the male prepuce and is done for medical, cultural, or religious reasons (97). It is one of the most common surgical procedures in urological practice (93, 98). Circumcisions are recommended by WHO to prevent HIV transmission in high-endemic countries and should be performed by well-trained health personnel (99). The most frequent reason for medical circumcisions is phimosis (93, 94).

Studies of circumcision in infancy have shown it to be protective against invasive penile cancer. Larke et al. showed in a systematic review a summary odds ratio of 0.33 (95% CI 0.13-0.83) for circumcision in childhood/adolescence and risk of invasive penile cancer. For men circumcised mostly as adults, data pointed at a reversed relationship, but this was not significant (OR 2.71 (95% CI 0.93-7.94)). A sensitivity analysis in three studies on childhood/adolescent circumcision as protection against PeIN was not statistically significant, but showed a trend of decreased risk for PeIN (OR 0.77 (95% CI 0.54-1.11)) (100).

In Israel, the incidence of penile cancer is very low, at 0.1/100 000 men and they also have a high incidence of circumcision in childhood, argued to be part of the explanation for the low incidence (3). Interestingly, the prevalence of genital warts among men in Israel is similar to other Western countries such as the United Kingdom, France and the United States, but unfortunately data on HPV prevalence in men in Israel is scarce (101).

Circumcision reduces the risk of genital HPV infection in men and reduces the risk of cervical cancer in women with HR sexual partners (men who had had ≥ 6 lifetime sexual partners and who had their first intercourse before the age of 17 years) (102).

In a systematic review by Larke et al., circumcised men were less likely to have prevalent genital HPV infection compared to uncircumcised men with an OR of 0.57 (95% CI 0.45-0.71) (103). A recent RCT confirmed that circumcision prevented HPV, with a 40% lower HPV incidence and a 35% lower HPV reinfection over 24 months in the circumcised group compared to the control group (66).

Immunosuppression and organ transplantation

Immunosuppression is mandatory after organ transplantation and is a known risk factor for non-melanoma skin cancer (NMSC) (104-106).

Madeleine et al. have shown that organ-transplanted patients have an increased SIR of 18.6 for PeIN and 3.9 for invasive penile cancer (107). Data on the risk of developing invasive penile cancer or PeIN with the new immunosuppressive therapies with biological treatments, used to treat psoriasis, rheumatological joint diseases and inflammatory bowel diseases does not exist, except in one case report on PeIN following treatment with Adalimumab (108).

A meta-analysis investigating risk for cancer in patients with HIV/AIDS found a SIR of 4.42 (95% CI 2.77-7.07) for penile cancer (109). A recent population-based study on 22 623 HIV-positive people (70% were MSM) diagnosed between 1990 and 2010 in San Francisco showed an increased risk of penile cancer, with a standardised incidence ratio of 3.8 (95% CI 1.4-6.1) compared to a reference population (110).

PUVA treatment

Psoralen and ultraviolet light (PUVA) is a treatment for skin diseases, and before the era of biological treatment options it was often used to treat psoriasis. PUVA treatment is a known risk factor for NMSC (111). An increased risk of penile cancer after PUVA treatment has been shown by Stern et al. with an incidence rate ratio of 4.5 (95% CI 1.3-16.1) (112).

Socioeconomic factors

Data from USA show that penile cancer is diagnosed in later stages in black and Hispanic men compared to white men, due to socioeconomic factors and inferior access to health care (7). In Sweden, Torbrand et al. have investigated socioeconomic factors and penile cancer and found that low educational level, low disposable income and being single were risk factors for invasive penile cancer, but no associations were seen for PeIN (113).

In Denmark a population-based study found an increased risk of invasive penile cancer in single men compared to married men. Numbers of sexual partners were not known. Data on PeIN was not shown (12).

Smoking

Smoking is a well-known risk factor for many cancer types. Hellberg et al. calculated a 4.5% increased risk of penile cancer in smokers in 1987. The dose relation between smoking and penile cancer was shown to be 1.88 (95% CI 1.10-

3.19) for men smoking > 10 cigarettes/day (90). Harish & Ravi studied smoking in 503 penile cancer patients and in 503 age-matched controls showing a significant association with smoking, OR 1.44 (95% CI 1.12-1.86) (114).

Daling et al. showed in 2005 that a greater proportion of men with penile cancer were current smokers compared to controls, 35% and 21.8% respectively, with an adjusted OR of 2.3 (95% CI 1.4-4.0) (95). No correlation between current smoking and PeIN was shown.

Sexual orientation

Studies on penile cancer and PeIN have not focused on separating incidence data or data on risk factors due to sexual orientation. No study was found investigating penile cancer in MSM, bisexual men or transgender persons. In a study by Kreuter et al. PeIN II and III were found in 3% (8/263) of HIV-positive MSM (115).

Diabetes

A recent register study from Denmark is the first to study diabetes and the risk of penile cancer and PeIN. They found an increased incidence rate ratio of 1.5 (95% CI 1.2-1.9) for having penile cancer in men with diabetes. Due to the register-based study design, they were able to adjust for age and educational level, but not other life-style related risk factors such as BMI and smoking. No statistically significant risk of PeIN in men with diabetes was seen.

Reinholdt et al. hypothesised that the increased risk for penile cancer is due to either oxidative stress and DNA damage through hyperglycaemia, dysregulated cellular immunity due to poor glycaemic control leading to infections, or increased risk of phimosis in men with diabetes (116).

Overweight and obesity

Overweight and obesity in patients with penile cancer is not well-studied. Only one study, by Barnes et al., was found, showing invasive penile cancer cases to be more overweight (BMI 25-29.9) than controls with an OR of 2.64 (95% CI 1.81-3.86), and more often obese (BMI >30) with an OR of 3.24 (95% CI 2.07-5.08).

They hypothesised that the increased risk for penile cancer was due to physical phimosis caused by overweight, increased prevalence of diabetes leading to phimosis and systemic effects such as chronic inflammation, oxidative stress and insulin resistance. Unfortunately, the study design did not include data on confounding factors (117). No study was found investigating BMI in patients with PeIN.

Hygienic hypothesis

Previously, there was a hygiene hypothesis, declaring that not washing the penis properly and thereby accumulating smegma could result in an increased risk of penile cancer. Van Howe debunked this myth in 2006 by reanalysing studies in both animals and humans without finding any scientific evidence supporting smegma as carcinogenic in penile, cervical or prostate cancer (118).

Other risk factors

Other known risk factors for NMSC such as UV light from outdoor sun exposure, solariums or treatment with narrow-band ultraviolet B (UVB) have not been systematically investigated in penile cancer.

In NMSC some professions are at greater risk of SCC due to exposure to UV light or exposure to chemical substances such as arsenic. Occupation in relation to penile cancer has not been well studied. In a study by Wesseling et al. cancers in workers on banana plantations in Costa Rica had a SIR for penile cancer of 149 (95% CI 55-324), though based on only six cases.

The increased risk for penile cancer could be due to sexual and socioeconomic factors but Wesseling argues that dermal contact with pesticides as a risk factor for penile cancer should be considered (119). In another study by Wessling et al. the relative risk for penile cancer in men with high pesticide use versus men with low pesticide use was 1.05 (95% CI 0.57-1.93) (120).

Graham et al. studied the concentration of arsenic in Bowen's disease (SCC in situ) and showed significantly higher concentrations of arsenic in SCC in situ lesions compared to controls, and argued for an etiological role in cancerogenesis. No predilection of any specific location on the body was noted (121).

Studies on other potentially cancerogenic chemical substances in food or water in relation to penile cancer were not found.

Many cancers including SCC have hereditary forms, but data on penile cancer and heredity could not be found.

Progression of PeIN into invasive penile cancer

Data on how often PeIN develops into invasive cancer is scarce. According to older studies with relatively few cases, transformation from PeIN to invasive cancer was calculated to occur in 10 to 30% of cases (122-125). It was argued by Wieland et al. that PeIN localised on the glans or inner prepuce has the highest level of transformation to invasive cancer (123).

Newly published data in 380 PeIN cases from the Netherlands, showed malignant progression of PeIN to invasive penile cancer in 7% of cases (26/380) during a median follow-up of 52 months (Interquartile range (IQR) 23-85 months) (17). The classification of PeIN used in the study by Hoekstra et al. was the former histologically used grading into PeIN I, II and III. When separated, malignant progression in PeIN I was seen in 2% of cases (1/42), in PeIN II in 8% (7/84) and in PeIN III in 7% (18/254) (17).

Another recently published paper is a study by Ashley et al. in 137 PeIN cases, where 13.6% progressed to invasive cancer within 29 months (82). They showed a similar proportion of progression into invasive cancer regarding P16-positive and P16-negative PeIN cases. No further data was found on the malignant progression according to the new classification of PeIN into *differentiated* and *undifferentiated* subtypes.

However, in VIN, Thujis et al. have shown a 10-year cumulative risk for vulvar SCC of 9.7% in HSIL and 50% in *differentiated* VIN (126). This is in accordance with HPV in vulvar SCC showing a more favourable outcome than non-HPV-related vulvar SCC (127). Further studies of malignant progression of *undifferentiated* and *differentiated* PeIN are needed to see if it correlates with vulva and with the known prognostically favourable outcome in HPV-positive penile cancer (128, 129).

Treatment

Treatment of penile cancer

Historically, surgery for penile cancer has been mutilating, often including a total penectomy, but studies have shown that organ-sparing surgical techniques can be used in PeIN, T1, T2 and selected cases of T3 tumours (130-132). When surgical organ-sparing techniques are used, patients quality of life and sexual function improve (133). Organ-sparing techniques for invasive cancer mean surgical excisions with minimal clinical-free margins outside the tumour, including wide local excision (WLE), circumcision, glansectomy with or without a reconstruction of the glans with split skin graft from the thigh. In more advanced penile cancer cases (T3 and T4) partial and total penectomy is needed to eradicate the penile cancer (130, 132).

Guidelines in the USA, Europe and Sweden recommend organ preserving procedures for local penile cancer and PeIN (130, 132, 42). Studies show that centralised treatment of penile cancer in larger centres favour the use of penile preserving treatments with a reduced mortality (131, 134). Penile preserving treatment options show a higher frequency of recurrence, but mostly local recurrences that do not influence the overall survival rates (130, 135-137).

Organisation of penile cancer care in Sweden

The Swedish National Penile Cancer Register (NPECR) was founded in 2000. In September 2013, a national multidisciplinary team conference (MDC) was launched and still continues every week. All new and recurrent penile cancer cases are presented and discussed in the MDC. Participating specialists include urologists, oncologists, dermatologists, pathologists and radiologists.

In 2015, the national penile cancer care was centralised into two main centres performing all the penile surgery of invasive penile cancer, and responsible for the digital MDC. Treatment of PeIN lesions is not centralised and is performed by both urologists and dermatologists.

In 2017 a national standardised care process was implemented for invasive penile cancer, with targeted lead times to optimise and equalise the penile cancer treatment and care (42, 138).

Treatment of PeIN

In PeIN, in addition to WLE and circumcision, recommended organ-sparing techniques also include total glans resurfacing (TGR), Mohs micrographic surgery, laser treatment, photodynamic therapy (PDT), curettage, electrocautery, diathermy and application of topical imiquimod or topical fluorouracil (5-FU) (130) (Table 3).

Table 3.
Treatment of PeIN, clearance rates and recurrence rates. Note the different time relapses for follow-up and limited data for some treatment methods (see text).

Treatment	Clearance rate %	Recurrence rate %	Follow-up time in months
WLE (47)	Free margins not presented	15-20%	Median 40 (26-65.6)
Circumcision (47)	Free margins not presented	15-20%	Median 40 (26-65.6)
TGR (139, 140)	Initial positive margins in 3.8-48%	4-11.5%	Median 38 (13-86)
Mohs micrographic surgery (141)	100% intraoperatively	0.84%	Median 40 (no data on IQR)
Laser surgery (47, 142-145)	96%	10-48%	Median 63.8 (20.9-95.2)
Topical 5-FU (146, 147)	73%	20%	Mean 34 (12-180)
PDT (142, 148)	67-83%	0%	Mean 18 (8-30)
Topical imiquimod (149)	57-63%	4%	Mean 11.4 (1-48)
Diathermy	Small case series	Not studied	-
Curettage	Case reports	Not studied	-
Electrocautery	Case reports	Not studied	-
Cryotherapy	Clinical experience	Not studied	-

WLE=wide local excision, TGR=total glans resurfacing, PDT=photodynamic therapy, IQR=interquartile range

Since data on treatment of PeIN from randomised controlled studies are lacking, treatment guidelines are based on small studies and case series (125, 130, 132, 150). The most common treatment used in PeIN in 49-85% of cases is surgical treatment, with circumcision as the mainstay. Minimal margins of a few millimetres are considered a sufficient treatment (38, 136, 147, 151).

In surgical treatment of PeIN, a risk of recurrence is reported in 15-20% of cases (47). Small PeIN tumours on the glans can be excised with WLE, but in more extensive PeIN on the glans TGR can be required. TGR implies that the skin of the glans is excised and replaced by a split skin graft from the thigh, with a good cosmetic and functional outcome (130, 152).

Follow-up after TGR and partial glans resurfacing in 25 PeIN cases showed positive margins in 48% of cases of whom 28% underwent further surgical interventions. Reported recurrences were 4% (mean follow-up for 24 months) (139). In a recent study of TGR in 26 patients (42.4% PeIN lesions and 53.8% T1 tumours) positive margins were seen in one case (3.8%) and that patient had a T2 tumour and underwent glansectomy and lymph node staging. A median follow-up of 38 months (IQR 13-86) showed a one-year recurrence-free survival of 96.1% and a two-year recurrence free-survival of 88.5% (140).

Mohs micrographic surgery, an organ-sparing technique that uses comprehensive microscopic margin evaluation to confirm complete tumour removal before reconstruction, has in previous studies been found to be time-consuming, expensive and with a relatively high recurrence rate (125).

However, a recent study by Lukowiak et al. retrospectively reviewed 119 invasive skin cancers of the penis treated with Mohs micrographic surgery with a risk of recurrence of only 0.84% and with a mean follow-up time of 3.25 years. The authors did not address the time consumed and the expense of the surgical method. They argued that since patients reported high satisfaction with sexual and urinary function and due to the low recurrence rates, more men with genital skin cancers may benefit from Mohs micrographic surgery and it may be indicated for tumours that are not currently included in treatment guidelines (141).

Another organ-sparing treatment within the surgical field used to treat PeIN is laser excision and/or vaporisation with ablative carbon dioxide (CO2) laser or neodymium:yttrium-aluminium-garnet (Nd:YAG). Several studies exist showing clearance of the PeIN in up to 96% of cases. Recurrence rates varied between 10% and 48% (47, 142-145). The great variance in recurrence rates between the studies could be explained by different lasers used, the completeness of the method used depending on the performer, and different lengths of follow-up time.

A treatment option within the topical field is PDT, where a photo sensitising cream is applied to the PeIN, the area is covered for three hours and then the lesion is treated with a specific light source. Fai et al. studied treatment with PDT in 23

patients with PeIN, showing a complete clearance in 83%. No recurrences were seen during follow-up of in mean 18 months (8-30 months) (148).

A review by Maranda et al. of 67 patients with PeIN treated with PDT, showed a complete clearance in only 67% of cases. Recurrence rates were not investigated (142).

Two topical cream treatments are used to treat PeIN. The first one is imiquimod, an immunomodulating cream also used to treat genital warts, actinic keratosis and superficial BCCs. In a small review by Mahto et al., in 27 patients the complete clearance of PeIN (including 11 cases of BP) treated with imiquimod was 78% (21/27). Duration of follow-up was in mean 7.5 months, varying between one and 22 months, but with no data on rate of recurrences (153).

A more recent review by Deen & Burdon-Jones regarding imiquimod as a treatment for PeIN (including eight cases of BP), showed complete clearance in 63% (30/48) of cases. Follow-up was in mean 11.4 months (range 1-48 months) with 4% (2/48) of reported recurrences (149).

The other cream is topical 5-FU, a topical chemotherapy preparation, also used to treat actinic keratosis, extra genital SCC in situ and superficial BCCs. Topical 5-FU showed a complete clearance in 73% of PeIN cases (146). In another study with 5-FU as the first line treatment and imiquimod as the second line treatment, complete clearance was seen in 57% (25/44) of PeIN cases and a partial response in an additional 13.6%. Recurrences were seen in 20% of cases (147).

A recent study of topical treatment in PeIN, using topical 5-FU in 17 patients and imiquimod in three patients showed complete clearance in 65% of cases (13/20) and partial clearance in 25% (5/20). Median recurrence-free survival was 14 months and 50% (10/20) needed further treatment (154).

Cryotherapy with liquid nitrogen is extensively used to treat extra genital SCC in situ, with complete clearance in 67% of cases, as shown in an RCT by Morton et al. (155). Cryotherapy has been used in the clinical setting to treat PeIN, but no study exists.

Another common treatment for extra genital SCC in situ is curettage and diathermy or electrocautery, but only small corresponding studies on complete clearance in PeIN exist (40, 150).

Recommended treatment

Since PeIN is an uncommon pre-cancer, no RCT exists on which treatment to choose. In the national MDC in Sweden the recommendations based on European Association of Urology (EAU) guidelines and Swedish national guidelines (130, 42) recommend circumcision when the PeIN lesion is located on the foreskin, since it is

a common and easy surgical procedure, performed under local anaesthesia with few complications.

If the PeIN is small, placed in any location, surgical WLE is usually recommended by the national MDC to get a histological assessment of microscopic margins.

Recommended treatment by the MDC of PeIN involving the urethra usually includes surgical excision with a cone, to minimise metastatic spread and because the lesion is harder to assess clinically.

Topical treatments are suggested by the MDC when the PeIN lesion is located on the glans or has a multifocal appearance. Topical 5-FU is recommended as the primary topical treatment and imiquimod as the second line, or sometimes in *undifferentiated* PeIN in younger patients as the primary topical treatment.

If the PeIN lesion is not cleared or recurrent, MDC recommends treatment with excision or TGR. The ability to administer topical treatment and the opinion of the patient is always considered in the recommendation given.

An interesting note is that in the vulva the recommended treatment for *differentiated* VIN is always surgical excision due to the higher risk of becoming an invasive vulva cancer compared to HSIL (156-158).

Prognosis in penile cancer

Localised penile cancer tumours have an excellent outcome, but loco-regional and metastatic diseases remain a fatal disease with shorter overall survival (159). The overall five-year relative survival rate in Sweden is 82% (1). The age-standardised mortality rate has been decreasing in Germany and the overall five-year survival between 2003 and 2012 was 72.4% (95% CI 64.8-80.0) (10). Hansen et al. studied five-year survival for penile cancer in Norway between 1956 and 2015 and showed a stable five-year survival of 61.6%, and unlike in Germany, an increasing mortality (11).

The most important prognostic factor in penile cancer is lymph node metastasis. Another prognostic factor is lymph node metastasis with extra nodal extensions (130, 160-162).

Both in oropharyngeal and vulvar squamous cell carcinomas, HPV positivity has been shown to be prognostically favourable. In squamous cell carcinoma of the penis, earlier studies regarding survival in relation to HPV status show conflicting results (163-165). A recent study by Chu et al. of 226 penile cancers showed HPV in 32.7% of penile cancers (74/226) with a survival benefit in HPV-positive tumours compared to HPV-negative tumours (129).

A prognostically favourable outcome has also been shown in a systematic review and meta-analysis by Sand et al. investigating 20 studies with a total of 649 men with penile cancer. They were tested for HPV or P16 and HPV-positive or P16-positive tumours showed a significantly favourable disease-specific survival (128).

Contrary to this, basaloid penile cancer has been shown to be more prone to metastasise (125, 166, 167), despite usually being HPV-positive, which would be expected to be a prognostically favourable state.

A recently published study by Wang et al. evaluated the 8th edition of the American Joint Committee on Cancer's Staging System for penile cancer and showed better discriminative ability for prognostic stratification compared to the 7th edition. Adding HR HPV status further improved the prognostic stratification in patients with node positive disease (168).

Urethral involvement has been shown to be an independent predictor of overall mortality on multivariate analysis (169). This is in accordance with vulvar squamous cell carcinomas located on the clitoris, which have worse survival prognosis compared to vulvar SCC elsewhere, argued to be due to unfavourable histopathologic characteristics such as the larger diameter of the tumour, a deeper degree of invasiveness and a higher percentage of positive lymph nodes, rather than the location (170).

No data was found regarding overall survival in patients with PeIN, since PeIN is a penile SCC in situ with no metastatic potential if not becoming an invasive penile cancer first.

Recurrence rates and follow-up

In a retrospective study of 700 penile cancer cases, 74% of the recurrences were discovered during the two first years of follow-up. During the first two years, as regards distribution, 66% of the local recurrences, 86% of the regional recurrences and 100% of the distant metastases were discovered. After five years of follow-up, 92% of all recurrences were discovered. After five years, only local recurrences or new primary tumours were seen (135).

Based on these data, both the European guidelines and the national Swedish guidelines regarding penile cancer and PeIN recommend follow-up for five years (130, 42).

Aims

The studies upon which this thesis is based were designed to fulfil the following aims:

- To investigate risk factors, incidence over time, and given treatment over time among patients diagnosed with PeIN (studies I and III).
- To determine the prevalence of HPV types and histopathological diagnoses in symptomatic circumcised preputium (study II), and
- To determine the prevalence of HPV types in invasive penile cancers compared to age-matched controls, and for HPV16, to measure copy numbers and viral activity (study IV).

Materials and methods

National Penile Cancer Register

In year 2000, the Swedish NPECR was founded. In Sweden, it is mandatory by law to report all penile cancers to the Swedish Cancer Register (SCR), a nationwide register established in 1958. To assess the coverage of PeIN cases in the NPECR, the SCR was used as a comparison. Between 2000 and 2017, the coverage was 99-100% in the NPECR compared to 100% in the SCR. In 2018 the coverage was 96% and in 2019 91%, due to a lag in registration by doctors.

The steering committee of the NPECR regularly improve the register, resulting in new variables being added over the years. Information about complications and whether the diagnosis was based on histological or clinical examination was added in 2009.

The register does not contain any information on follow-up of PeIN cases and thereby does not contain information on recurrence rates or treatment outcome for PeIN. There has been discussion about adding it, since it is very valuable information, but it has not yet been included. The apprehension of the steering committee of the NPECR is that data on follow-up of PeIN would be incomplete since PeIN cases are followed by different medical specialists such as urologists and dermatologists, and also followed in different clinics both in hospitals and private practices.

Penile Cancer Data Base Sweden

The steering committee of the NPECR generated a database with the Swedish Regional Cancer Centre in 2013 called the Penile Cancer Data Base Sweden (PenCBaSe). In PenCBaSe, data from the NPECR from 2000 to 2012 was linked to data from the National Inpatient Register (IPR), the National Outpatient Register (OPR), the SCR, the Cause of Death Register, the National Register of Prescribed Drugs (RPD), the Register of the Total Population and the Longitudinal Integration Database for Health Insurance and Labour Market Studies.

The IPR was founded in 1964 and contains information on inpatient care, with coverage of the whole of Sweden since 1987.

The OPR was founded in 1997 and contains information about outpatient surgeries. Since 2001, the OPR has also included information about all hospital-based outpatient physician visits.

All drugs prescribed in Sweden that patients obtain from the pharmacies are registered in the RPD, founded in 2005. The purpose of the PenCBaSe database was to facilitate research on penile cancer in Sweden.

All cases of penile cancer and PeIN in the NPECR between 2000 and 2012 were included in the PenCBaSe, and for every included case, six controls were randomly chosen from the Register of the Total Population. The controls were free of penile cancer and matched on age and county of residence.

In study I, data on all PeIN cases and their matched controls in the PenCBaSe were included.

In study III, data from the NPECR from 2000 until the end of 2019 was used, with the data extraction made on the 7th of December 2020.

Questionnaire

A questionnaire regarding medications, smoking habits, number of lifetime sexual partners, former diseases and surgery performed on the penis were created by our group according to potential risk factors investigated in study I (see supplementary 1 in Paper II for English version). The questionnaire was written in Swedish and completed by the included patients in study II, and by the included cases and controls in study IV.

Ethics

All studies were approved by the ethics board in Lund, with diary number 2015/907, including two amendments with diary number 2016/556 and 2017/839.

All included patients in studies II and IV signed a written informed consent. In study IV, cases were included after they had their penile cancer operation, resulting in a few cases where the patient unfortunately died before inclusion. In these cases, the patients' life partners signed the informed consent.

HPV analysis

In study II and in the non-malignant controls in study IV an approx. 5 mm biopsy was cut from the foreskin during the surgical procedure and immersed in RNA laterTM (Invitrogen, Thermo Fisher, Vilnius, Lithuania).

After transportation of the biopsies to the Department of Microbiology in Lund, they were transferred to 1 mLGITS-solution (4 m guanidinium thiocyanate, 22 mm NaCitrate and 5% Sarcosyl [N-Lauroylsarcosine sodium salt] and 1% mercaptoethanol) and incubated at room temperature overnight. Extraction of the DNA was performed with the total NA-kit (Roche, Stockholm, Sweden) using MagNA Pure LC (200 µL input and 100 µL output).

The adequacy of the sample was assessed by testing 5 µL of the sample for the human b-globin gene with real-time PCR (171).

Modified general primer PCR (MGP-PCR) was used in a simultaneous identification of 40 genital HPV types in a 25 µL reaction, containing 5 µL of extracted material (172, 173). Subsequently a Luminex analysis was performed with probes for the 40 genital HPV types shown here: 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68 (a and b), 69, 70, 73, 74, 81, 82, 83, 85, 86, 87, 89, 90, 91, and 114.

In study IV, the HPV16-positive samples were further analysed to determine copy numbers and whether the virus had episomal or integrated DNA. The integration of HPV DNA into the human genome most frequently disrupts the E2 gene (174). As a surrogate marker for the physical status of HPV16, the quantity of HPV16 E2 gene was determined as described by Letsolo et al. (175). Here, we used the mean log10 values of E2 and E7 copy-numbers from each sample and calculated the ratio of E2/E7 gene copy-numbers of HPV16 to investigate the presence of integrated, mixed and episomal forms of HPV16.

HPV16 was classified as integrated when no E2 copy numbers could be detected and E7 copy numbers were present, mixed status when E2/E7 ratios were 0.1-0.8, and episomal status when the E2/E7 copy number ratio was >0.8. The samples were analysed in duplicate.

In addition, another aliquot (200 µL) of the GITS-lysate, was used for mRNA extraction using the Oligotex Direct mRNA Mini Kit (Qiagen). The extraction was performed according to the manufacturer's protocol for isolation of PolyA mRNA from animal tissues. The mRNA was eluted by adding 45 µL of Oligotex elution buffer (70°C) to the column and centrifuging for 1 minute at maximum speed. Purified mRNA was stored at -80°C until use.

Quantitative PCR of HPV16 E7 mRNA was analysed in triplicate and performed as previously described (175). To compensate for the smaller elution volume of the

mRNA extraction (45 µL) compared to that of the DNA extraction (100 µL), the HPV16 mRNA copy numbers were divided by 2.5. The HPV16 mRNA expression level was given as HPV16 mRNA copy numbers per HPV16-DNA copy.

Histopathological examination

In study II, clinical diagnosis and visible skin changes were derived from the medical record. Since not all included patients had phimosis, but all had symptoms or problems related to the preputium, all cases were entitled symptomatic foreskin. The excised foreskin was sent for routine histopathological examination with diagnoses performed on hematoxylin and eosin (H&E) stains.

In study IV, two experienced pathologists with a sub-specialisation in uropathology retrospectively reviewed the histopathological examination of subtype of penile cancer, blinded to the primary diagnosis. One of the participating pathologists was assigned to choose one H&E stained representative slide for each tumour. The assessment of the subtype of penile cancer was made on glass slides in 106 cases and by using a high-resolution digital slide in 40 cases. Nanozoomer S360 (S60 for large histologic sections) by Hamamatsu and software Sectra IDS7 were used for scanning.

When tumour histology and tumour grade were assessed, HPV status was unknown to the pathologists, to avoid bias. After individual assessment by the two pathologists, the diagnoses were compared between the pathologists and discrepancies were found in six cases. To be able to make a final diagnosis, p16INK was used in one case and HPV PCR in five cases and then the final subclassification could be agreed upon.

The histopathological examination of the diagnoses in the non-malignant controls was routinely made using H&E stains in the same manner as in study II.

Statistical analysis

Study I

In this case-control study, conditional logistic regression was used to compare the risk of PeIN with selected risk factors. Crude odds ratios (OR) were calculated, and then adjusted for educational level, marital status and comorbidity. Statistical analysis was performed using the R programme package (3.4.3) by the R Core Team.

Study II and study III

Chi-square tests were used to calculate differences between groups such as cases with or without HPV. When $p \leq 0.2$ the statistical analysis continued with logistic regression. A multivariable logistic regression was performed to adjust for age and smoking. The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY). In study III, the age-standardised incidence and 95% Confidence Intervals (CI) were calculated in Stata SE 16.1 and the population at risk was standardised according to the latest European Standard Population (ESP) from 2013.

Study IV

As in studies II and III, statistical analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY). Correlation was calculated with Chi-square tests, and Fisher's exact test in small numbers. When $p \leq 0.2$ OR were calculated with multiple logistic regression adjusted for > 10 sexual lifetime partners, smoking, former smoking, former skin disease, former phimosis, former penile biopsy, former penile surgery and former genital warts. Cases and controls were not analysed as paired cases.

Results

PeIN – risk factors, incidence over time and treatment over time (study I and III)

In the series of 580 PeIN cases as compared with 3436 controls (study I), more cases with PeIN had diseases of the prepuce, balanoposthitis, genital warts, LS, LP, took immunosuppressive drugs, were organ-transplanted and had previously gone through penile surgical procedures ($p < 0.001$). Obesity was more common in PeIN cases (1.6%) than controls (0.7%) p=0.003. No statistically significant differences were found for smoking, tobacco use or Chronic Obstructive Pulmonary Disease (COPD).

Increased adjusted OR for PeIN was seen in men with diseases of the prepuce, balanoposthitis, genital warts, LS, LP, taking immunosuppressive drugs, who had been organ-transplanted or who had previously undergone surgical procedures on the penis (Figure 10).

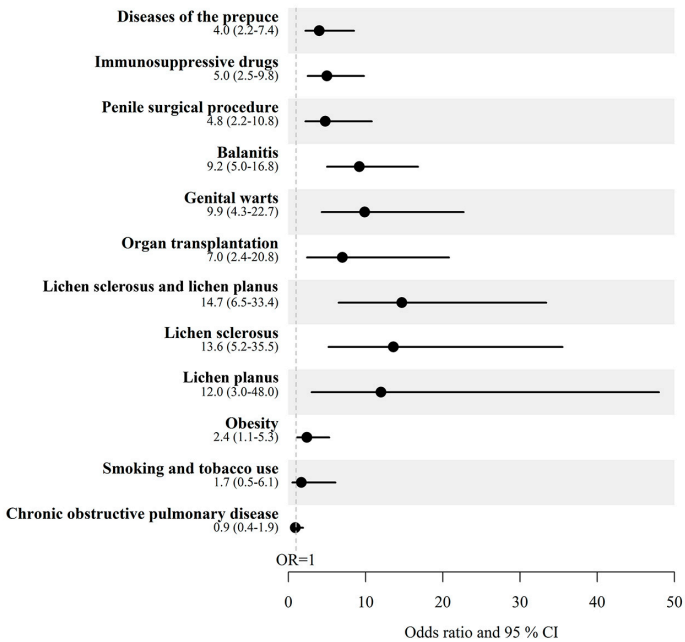

Figure 10
Forest plot showing OR for the different diagnoses and procedures on the penis.

Study III comprised 1113 men diagnosed with PeIN in Sweden between 2000 and 2019 and showed an age-standardised incidence of PeIN with 1.40/100 000 men (95% CI 1.32-1.49). The standardised incidence rate was 2.37 (95% CI 1.56-3.70) in 2019 compared to the baseline incidence in 2000 (Figure 11).

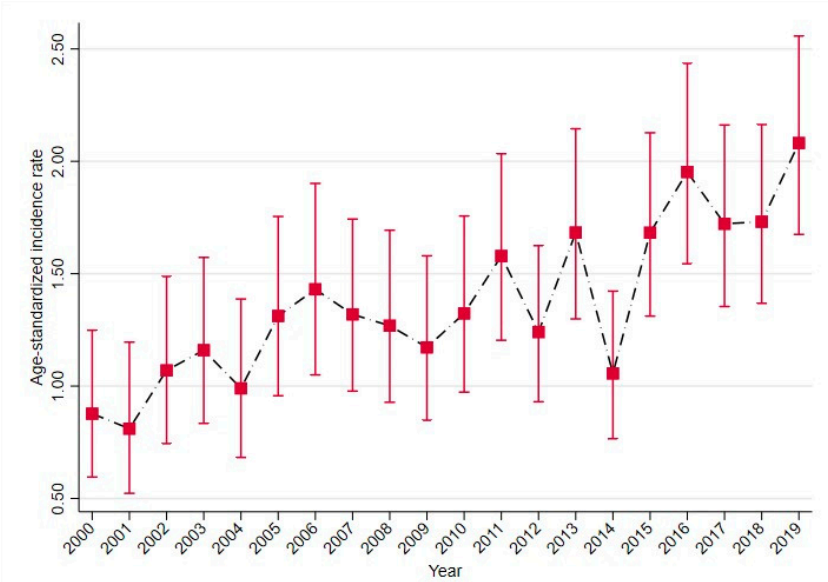

Figure 11
Age-standardised incidence of PeIN in Sweden between 2000 and 2019.

In 75% (835/1113) of PeIN cases, treatment given was surgical and in 14.6% (163/1113) of PeIN cases treatment given was topical. The number of PeIN cases treated with surgical and topical treatments in relation to age, size and localisation of the PeIN, complication rate and clinical or histologically diagnosed PeIN were calculated.

Comparisons between treatment groups were made and changes in treatment given over time were calculated.

Results show local surgery to be more common than laser surgery in the last five years compared to the first five years of the period studied, OR 5.75 (95% CI 2.94-11.27). A higher risk of complications was seen in laser surgery compared to local surgery OR 2.82 (95% CI 1.10-7.19).

Regarding changes in topical treatments over time an increased OR of 9.48 (95% CI 2.29-39.24) was seen for having treatment with imiquimod or topical 5-FU compared to patients treated with either PDT, cryotherapy, diathermy, or electrocautery in the last five years compared to the first five years of the period studied.

HPV types and histopathology of circumcised preputium (study II)

The most common clinical diagnosis in the 351 included men was phimosis, seen in 85.2% (299/351). The second most common clinical diagnosis was visible skin changes, though without phimosis in 8.8% (31/351). By histological routine examination, LS was diagnosed in 58.7% (206/351) of cases, followed by lichenoid dermatitis in 9.1% (32/351). LP was seen in 5.7% (20/351) (Table 4).

PeIN was found in 2% (7/351) of cases despite no clinical suspicion of malignancy. Normal skin on histopathological examination was seen in only 13.1% (46/351).

Table 4.
Histopathological diseases in 351 cases of circumcised symptomatic preputium

Histopathological diagnosis	Number (%)
PeIN	7 (2.0)
LS	206 (58.7)
LP	20 (5.7)
Lichenoid dermatitis	32 (9.1)
Psoriasiform dermatitis	5 (1.4)
Plasma cell balanitis	5 (1.4)
Unspecific chronic inflammation	30 (8.5)
Normal skin	46 (13.1)
Total	351 (100.0)

HPV was analysed in fresh tissue of all cases revealing HPV in 17.1% of cases (60/351) distributed among 28 different HPV types, 15 HR types and 13 LR types. HR HPV types were seen in 9.1% (32/351) with HPV16 in 2.3% (8/351) (Figure 12).

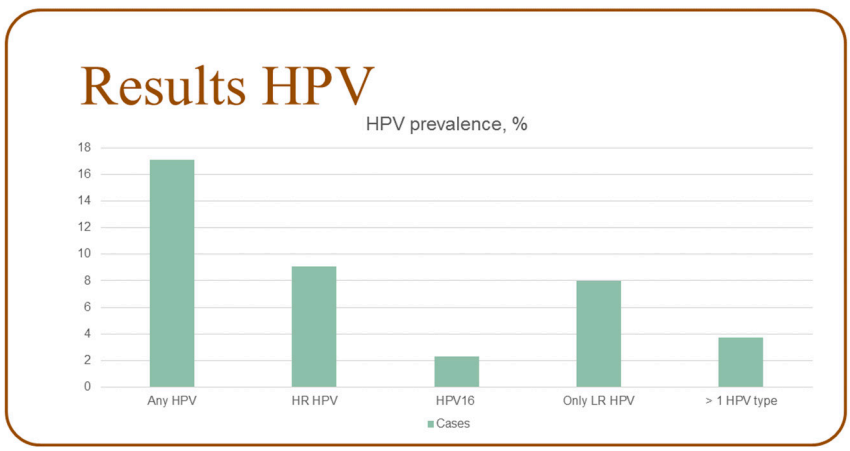

Figure 12
Distribution of HPV-types in 351 cases circumcised because of symptomatic foreskin.

HPV in penile cancer (study IV)

In this study, HPV was found in 38.5% (52/135) of fresh tissue in the tumour among 135 included penile cancer cases. HR HPV was seen in 34.5% (48/135) with HPV16 in 27.4% (37/135). Ten mm adjacent to the tumour samples showed HPV in 30.3% (40/132). Here, HR HPV was found in 25.8% of cases (34/132) with HPV16 present in 17.4% (23/132). The integration status of HPV was calculated in all HPV16-positive cases and showed the virus to be episomal in 59.5% (22/37), integrated in 27% (10/37) and mixed in 13.5% (5/37).

HPV16 mRNA expression showing viral activity was present in 86.5% (32/37) of the HPV16-positive tumours and in 21.7% (5/23) it was found 10 mm adjacent to the tumour. Mean viral copy/cell number was 74.4 (median 6.5, range 0.00003 – 725.4 viral copies/cell) in the HPV16-positive tumour compared to 1.6 (median 0.1, range 0.001 – 14.4 viral copies/cell) adjacent to the tumour.

HPV in penile cancer cases and age-matched non-malignant penile samples showed HPV in 38.5% (52/135) of the cases and in 11.4% (12/105), ($p<0.001$) of controls, with a crude OR of 4.9 (95% CI 2.4-9.7). Adjusted OR was 12.8 (95% CI 4.9-33.6).

HPV was most frequent in basaloid subtypes of penile cancer compared to other subtypes, seen in 100% (9/9) and 34.1% of cases (43/126) respectively, Fishers exact test $p<0.001$) (Table 5). HPV's second level of frequency was in warty subtypes, where it was seen in 88.9% of cases (8/9) versus 34.9% (44/126) in other subtypes (Fisher's exact test $p=0.002$).

Warty-basaloid subtypes of penile cancer were HPV-positive in 85.7% of cases (18/21) compared to 29.8% (34/114) in other subtypes ($p<0.001$) with an increased adjusted OR for HPV of 9.0 (95% CI 1.4 – 56.2, $p=0.019$). In the usual subtype of penile cancer, HPV was found in 13.6% of cases (11/81) ($p<0.001$). Decreased risk for HPV were seen in the usual subtype of penile cancer with an adjusted OR of 0.03 (95% CI 0.007 – 0.15, $p<0.001$).

Table 5
HPV types in the different subtypes of penile cancer and HPV in relation to histopathologic diagnosis in the non-malignant controls.

					HPV-types																	
Tumor subtype	**N**	**HPV+ (%)**	**Single HPV type**	**> 1 HPV type**	**6** LR	**16** HR	**18** HR	**31** HR	**42** LR	**43** LR	**51** HR	**52** HR	**56** HR	**66** HR*	**68** HR*	**70** LR	**73** HR*	**74** LR	**83** LR	**87** LR	**90** LR	**91** LR
Usual	81	11 (13.6)	10	1	1	3	1	2	1				1[a]		1[a]				1	1		
Warty/basaloid	21	18 (85.7)	18	0		15						1	1				1					
Basaloid	9	9 (100)	9	0		7		1			1											
Warty	9	8 (88.9)	6	2		6[b]	1					1[c]		1[c]	1[b]						1[b]	1[b]
Verrucous	5	0 (0)	0	0																		
Usual/basaloid	4	4 (100)	3	1		4[d]												1[d]				
Lymphoepitelioma-like	2	2 (100)	1	1		2[e]			1[e]													
Usual/verrucous	2	0 (0)	0	0																		
Papillary	1	0 (0)	0	0																		
Pseudohyperplastic	1	0 (0)	0	0																		
Total	135	52 (38.5)	47	5		37	2	3	2	0	1	2	2	1	2	0	1	1	1	1	1	1
Controls, histopatologic diagnosis																						
LS	63	7 (11.1)	6	1	1	1				1[f]				1				2[f]			1	1
LP	11	0																				
Lichenoid dermatitis	8	3 (37.5)	3	0											1			1			1	
Other	23	2 (8.7)										1										
Total	105	12 (11.4)	9	1	1	1				1		1		1	1			3			2	1

HR = high risk, LR= low risk. *HPV66, 68 and 73 were here classified as high risk, according to WHO they are potential high risk types (53).
[a, c, d, e, f] Samples double HPV positive for marked HPV types. [b]One sample was positive for 4 HPV types.

General discussion

Main findings

Risk factors for PeIN were studied in a case-control study of 580 cases and 3436 controls. Increased odds ratios were seen for LS and LP of 14.7 (95% CI 6.5-33.4), for genital warts, OR 9.9 (95% CI 4.3-22.7) and for balanoposthitis, OR 9.2 (95% CI 5.0-16.8). Even for taking immunosuppressive drugs, penile surgical procedures, and organ transplantation, increased ORs were demonstrated.

The incidence of PeIN was studied in the NPECR between 2000 and 2019, revealing an increased SIR of 2.37 in 2019 compared to 2000. Comparison of treatments given for PeIN showed surgery in favour of laser treatment and topical imiquimod and 5-FU in favour of local destructive methods such as PDT, cryosurgery, diathermy and electrocautery in the last five years compared to the first five years of the period studied.

Symptomatic foreskin was investigated in 351 cases, showing HPV in 17.1%, HR HPV types in 9.1% with HPV16 in only 2.3%. Histologically, LS, LP and lichenoid dermatitis was seen in 73.5% of cases and PeIN was found in 2% despite no clinical suspicion of malignancy.

Analysis of HPV in invasive penile cancer, 10 mm adjacent to the tumour and in non-malignant penile controls was performed, showing HPV in 38.5% and 11.4% of cases, respectively. HPV16 in the tumour was present in 27.4%. Viral activity in HPV16-positive cases was higher in the tumour (86.5%) compared to adjacent to the tumor (21.7%). The HPV16 virus in the tumour was episomal in 59.5% of cases and integrated in 27%. The most common HPV-positive subtypes of penile cancer were basaloid, warty and warty-basaloid subtypes, HPV positive in 100%, 88.9% and 85.7% of cases respectively.

Risk factors for PeIN

In study I, risk factors for PeIN were studied in a large case-control study of 580 cases and 3436 controls, revealing similar risk factors in PeIN as known risk factors for invasive penile cancer. Increased OR for PeIN in inflammatory skin diseases such as LS and LP were found, OR 14.7 (95% CI 6.5-33.4).

The risk of LS progressing into PeIN is up to 13.6% in LS cases described by Kravvas et al. with data derived from a clinic specialising in penile dermatology with 70% of referrals from urologists, dermatologists and genitourinary medicine (84). This highlights the need for diagnosing and treating LS, to prevent development into PeIN and subsequent penile cancer.

A study by Lee et al. investigating LS in women showed that long-term treatment with highly potent topical steroids improved symptoms, prevented scarring and removed cancer risk (86). No similar study in men exists but since morphologically it is the same disease in men and women, men would probably benefit from the same treatment and follow-up as women. In clinical practice many departments of dermatology have special vulvar clinics, but special penile clinics are seldom seen, so maybe penile clinics should be promoted to treat and follow-up men with penile conditions?

One limitation of the study was that HPV status was not known, hence the diagnosis of genital warts had to be used instead, showing an increased OR for PeIN of 9.9 (95% CI 4.3-22.7) pointing to the other main risk factor for penile cancer. Vaccination for HPV in boys has been included in the children's vaccination programme in several countries, including gender-neutral vaccination in Sweden, since 2020. In the school-based vaccination programme in Sweden, the nine-valent HPV vaccine is used, covering even the most common HPV types giving rise to genital warts. HPV vaccines have reduced the number of genital warts both in Sweden and in several other countries worldwide (75).

Smoking is a known risk factor for invasive penile cancer but could not be confirmed as a risk factor for PeIN in our study, probably due to the low number of participants and thus inadequate power. The reason for the low number of smokers was that doctors seldom record the diagnosis in the medical record and our study was based on diagnoses made by medical doctors. The same applies for BMI, so there are no statistically significant numbers, and no further conclusions can be drawn about increased BMI as a potential risk factor for PeIN.

The major strengths were the large study population, the case-control setting and that the diagnoses were made by doctors one year prior to the PeIN diagnosis.

One limitation of the study was the possibility that risk factors were misinterpreted and thereby missed as PeIN lesions, since histological confirmation was not always performed for all risk factor diagnoses. To minimise the risk of PeIN lesions being misclassified, a sensitivity analysis was performed and all risk factors one year prior to the PeIN diagnosis were excluded. Other limitations were the missing data on smoking, and BMI not allowing full evaluation of this data as a risk factor.

Incidence of PeIN

In study III the changes in incidence of PeIN over a period of 20 years, between 2000 and 2019, were studied in 1113 PeIN cases in Sweden and we showed a substantial increasing incidence. The increased incidence of PeIN highlights the importance of clinicians being aware of the symptoms and clinical presentation of the diagnosis, and the significance of educating medical students and doctors in more advanced levels of training about the symptoms and handling of the disease, despite PeIN being a rare disease. Since it is a rare disease, even medical doctors specialising in skin diseases, sexually transmitted diseases and urological diseases seldom see these patients and therefore need more education on PeIN and penile cancer.

The increased incidence of PeIN in Sweden shown here contrasts with the stable incidence in invasive penile cancer in Sweden between 2000 and 2012 (1). However, the data in our study includes more recent years, up to 2019. Since the development of invasive penile cancer takes many years, a lag in increased incidence of invasive penile cancer is expected. Increased incidence of invasive penile cancer has been seen in several European countries such as the UK, Germany and the Netherlands (8-10). Could it be that PeIN lesions are increasing in Sweden today and in the coming years, we will see an increasing incidence of invasive penile cancer?

Another explanation for the increase of PeIN lesions could be the sexual revolution in the sixties and seventies, with easily available contraceptives and the increased number of sexual partners, leading to extensive spread of HPV. HR HPV types are common in tonsil cancer, which has been shown to have increased in Sweden, and is argued to be due to increased HPV and subsequent malignant transformation after 20-30 years (176). Since HR HPV is common in PeIN lesions, one could expect the same process to occur on the penis, giving rise to the increased PeIN incidence seen in our study.

Another possible explanation could be a higher awareness among patients in recent years of the need to seek health care for symptoms of the genital area, which may not be considered embarrassing anymore. Even a higher awareness of potential cancers among medical doctors could be an explanation, due to a nationwide focus on cancers and the introduction of standardised care processes for many cancer forms, including invasive penile cancer. Another partial explanation could be the subspecialisation into two national penile cancer centres performing all penile cancer surgery and the introduction of national MDCs.

In cutaneous MM, a rapid increase in thin melanomas has been seen since 1975. Welch and colleagues argue that the reasons could be falling thresholds for pathologists in diagnosing melanomas, an economic incitement for dermatologists, where under-diagnosing can lead to penalties (but not over diagnosing) and more

money earned when more biopsies are performed and when patients must come for surveillance (177). This is an American context, but maybe this could be part of the explanation for the increased incidence of PeIN in Sweden as well?

An increase in the incidence of PeIN has been shown in the Netherlands and in Denmark, but the increase is much higher in Sweden, with an age-standardised mean incidence of 1.40/100 000 men compared to 0.47/100 000 men in the Netherlands (17). In Denmark, the age-standardised incidence rate of PeIN increased from 0.5 per 100 000 men-years to 0.9 per 100 000 men-years over a period of 11 years (19), which is lower than our age-standardised increase from 0.88/100 000 men in 2000 to 2.08/100 000 men in 2019. However, the period studied in Sweden is more recent, which could be a possible explanation for the higher increase seen in Sweden compared to in Denmark and in the Netherlands. Our results showing a substantial increased incidence of PeIN are further confirmed by a recent Danish study with incidence data up to 2018, showing an increased age-standardised incidence from 0.87 (95% CI 0.65-1.16) per 100 000 person-years in 1997-1998 to 1.84 (95% CI 1.55-2.20) in 2017-2018 (21).

The main strengths of our study are the age-standardised data allowing for comparison between populations differing in age distribution, and the extensive amount of data based on a national register with a high coverage and a total inclusion period of 20 years.

The main limitations are that the register did not hold data on treatment outcome and recurrence rates in PeIN.

Treatment of PeIN

In study III, treatments given for PeIN lesions in Sweden between 2000 and 2019 were investigated, showing surgical treatments to be used in 75% and topical treatments in 14% of PeIN cases.

Comparisons of changes in treatment methods used for PeIN lesions over time showed an increase in local surgery in favour of laser surgery, most likely due to data published by Chipollini et al. showing laser surgery to have recurrence rates of up to 48% compared to recurrence rates in WLE of around 20% (47). Our study also showed that topical treatments were used more often than locally destructive methods in recent years, probably due to studies showing total clearance in 74% of PeIN lesions treated with topical 5-FU (146) and a total clearance of 63% in PeIN treated with imiquimod (149) and the cosmetically favourable results of the treatment.

Another explanation could be that MDCs were introduced in Sweden in 2013 and also that an expert group produce and update national recommendations on

investigation, treatment and follow-up of penile cancer and PeIN, probably affecting treatment given. A better knowledge of the different potential treatments, a higher awareness of recent research, and adherence to international and national guidelines could be expected when penile cancer cases are discussed in the MDC.

Strengths and limitations of this study have been described under the heading Incidence of PeIN.

HPV types and histopathology of circumcised preputium

In study II, 2% (7/351) of PeIN lesions were found in the histopathology report, without clinical suspicion, strengthening the recommendation to send symptomatic excised foreskin tissue for histopathological examination, and in the case of PeIN, to offer further treatment if needed or follow-up according to recommendations in international guidelines.

LS, LP and lichenoid dermatitis were histopathologically diagnosed in 73.5% of cases in symptomatic foreskin. Inflammatory skin diseases are common explanations for symptoms of phimosis and skin disease. It could be argued that when the patient is circumcised, the problem is solved, and the disease treated. But this was proved to be wrong by Edmonds et al. who showed a clearance of the LS after circumcision in only 76% of cases (178). Kantere et al. also have shown that up to 64% of cases still have active LS after circumcision (179).

Without treatment of LS, the disease will progress with the risk of scarring, meatal stenosis, and in up to 13% of cases there is a risk of development into malignancy (84). This calls for awareness among doctors performing circumcisions of the need to inform the patient about the diagnosis and potential recurrence, and when ongoing symptoms occur, to follow-up or refer the patient for follow-up to a department experienced in penile dermatoses. Treatment guidelines for LS recommend the diagnosis should be confirmed and biopsy performed (180), which is in line with the experience resulting from our study revealing treatable lichenoid dermatoses in 73% of symptomatic foreskins.

In addition to making a diagnosis on the symptomatic, often narrow prepuce by sending circumcised preputial tissue for histopathologic evaluation, another argument for sending the tissue for histopathological evaluation could be patient orientation. When a skin disease on the genitals has to be confirmed by a biopsy, many patients are stressed due to embarrassment, concern about sexual function and fear of pain. So since only 13% of the cases included in our study had histologically normal skin, we can conclude that not sending the circumcised specimen represents a missed opportunity to make a diagnosis.

One argument for not sending the excised foreskin is the financial cost of the histopathology report, but since up to 64% of patients still have active symptoms after the circumcision (179), patients are likely to seek further health care, entailing a future cost for the society. In clinical practice patients tend to seek further health care several years after their circumcision, sometimes with severe scaring and complications such as meatal stenosis, causing an extensive impact on the patient's ability to urinate, sexual life and self-esteem. Medical measures then sometimes have to be more invasive, for example with dilatation of the meatus, resulting in a higher cost to the society and more suffering for the patient.

HPV prevalence in biopsies from fresh symptomatic foreskin was 17.1%, relatively low compared to topical swab tests from healthy men (50%) (65). The reason for our low HPV prevalence indicates the superiority of the sampling method representing HPV DNA mainly within the biopsied preputium. Furthermore, it has been observed that swab samples from superficial skin layers manifest substantially higher HPV prevalences than biopsies, indicating that HPV DNA is common in superficial layers but is not necessarily present throughout tumours (181).

Fifteen HR HPV types and 13 LR HPV types were found, with HPV16 being the most prevalent HR HPV type seen in 2.3% of cases. Vaccination for HPV in boys with the 9-valent HPV vaccine is safe and effective, is included in the school vaccination programme, and is likely to prevent progress to PeIN and penile cancer in the future.

Major strengths of the study were the high number of consecutively included and histopathology evaluated symptomatic foreskins with simultaneous analysis of a large number of HPV samples from fresh biopsies.

Limitations were that no standardised classification of the symptomatic prepuce was used before the circumcision and thus we were not able to correlate the amount of LS to clinical indications of LS pre-circumcision. Another limitation was that the median age of the included patients was relatively low, and that data were lacking on the lifetime tobacco exposure and occupation of the subjects.

HPV in penile cancer

In study IV, analysis of HPV in fresh biopsies of invasive penile cancer cases, showed an HPV prevalence of 38.5%. HPV16 was the most predominant HPV type, in concordance with earlier studies. We showed that the viral activity was higher in the tumour compared to 10 mm adjacent from the tumour, shown by a higher amount of mean mRNA.

Our HPV prevalence in penile cancer tumours is a little lower than in the pooled data of HPV (50%) from a meta-analysis of HPV in penile cancer by Olesen et al.

(52). This is probably due to the usage of fresh biopsies in our study, most probably closer to the true amount in the tissue compared to swabs, and with minimised risk of contamination. A recent study of frozen penile cancer tissue by Huang et al. found HPV in 38% (41/108) of penile cancer cases (59), similar to our HPV prevalence of 38.5%.

HPV16 was the most common HPV type in our study, in alignment with earlier studies. To our knowledge, this is the only study comparing HPV in fresh biopsies from penile cancer and fresh biopsies in non-malignant penile controls.

HPV16 viral activity measured by mRNA, was higher in the tumour (86.5%) compared to 10 mm next to the tumour (21.7%), suggesting that active HPV in the tumour is probably important for malignant transformation. The mean viral copy numbers of HPV16 were 74.4 copies/cell, similar to the median 72 copies per cell described by Heideman et al. (62). These numbers are low compared to the median viral load in vulvar cancer of 14 676 HPV16 copies/cell (182).

Most frequently, disruption of the E2 gene is involved in integration of HPV DNA into the human genome (174). In our study, any degree of integration of HPV16-positive cases was found in 40.5% of cases, much less than in Huang et al. who described integration in penile cancer in 92.1% of cases (59). The next-generation sequencing method used by Huang et al., seem to render a higher HPV integration rate than the E2/E7 method used by us, the latter suffering from the limitation of decreased detection of HPV integration when a large excess (at least 10-fold) of episomal HPV16 is simultaneously present (56).

HPV prevalence was highest in basaloid, warty and warty-basaloid subtypes, in accordance with earlier studies (52), though our numbers were slightly higher.

According to our HPV prevalence of around 38%, and with boys included in the national vaccination programme since 2020, we would expect that vaccination should prevent around 35% of future penile cancers.

The major strengths of this study were the case-control setting, the use of fresh biopsies to avoid cross-contamination (which can be a problem with paraffin-embedded samples), the revealing of the true prevalence of HPV in the tumour, the blinded sub-classification of the penile cancer subtype by two experienced pathologists, and the extensive analysis of HPV16 viral activity.

Limitations were a relatively high amount of missing information in questionnaires filled in by penile cancer cases, due to inclusion after surgery. Further limitations were the lack of full data on lifetime tobacco use in the questionnaire, the large extent of diagnoses of inflammatory skin disease among the penile controls, and the possible discrepancy between anatomic localisation of biopsies for the HPV sample in penile cancer cases compared to in controls.

Conclusions and future perspectives

Main conclusions

In conclusion, this thesis has investigated risk factors for PeIN in a case-control study, revealing increased OR for inflammatory skin diseases such as LS and LP, genital warts, balanoposthitis, taking immunosuppressive medication, former penile surgical procedures and organ-transplantation, similar to known risk factors for invasive penile cancer.

The incidence of PeIN in Sweden over a period of 20 years was explored, showing an increasing age-standardised incidence with a SIR of 2.37 in 2019 compared to the reference number of zero in 2000.

Treatments given for PeIN were examined, which showed 75% of cases were treated surgically and 14.6% were treated topically. Changes in treatment methods for PeIN over time were compared showing that local surgery and topical treatments were more common than laser surgery and local destructive methods in the last five years compared to the first five years of the period studied.

In circumcised symptomatic prepuce HPV was found in 17.1% of cases with HR HPV in 9.1% and HPV16 present in 2.3%. Histopathological examination showed 2% of cases had PeIN despite no clinical suspicion of premalignancy and 73.5% of cases had inflammatory skin diseases with LS being the most predominant, potential precursors of PeIN.

In conclusion, the study of HPV in fresh biopsies from penile cancer tumours and 10 mm adjacent to the tumour and in non-malignant penile controls revealed HPV in 38.5%, 30.3% and 11.4% of each biopsy localisation respectively. HPV16 was the most predominant HPV type. Viral activity and mean viral copies/cell were higher in the tumour compared to 10 mm adjacent to the tumour. The main status of HPV in the tumour was episomal (59.5%), whereas 27% of HPV16-positive tumours had fully integrated HPV DNA.

Clinical implications

The increasing incidence of PeIN highlights the importance of medical doctors having knowledge about the disease, and when clinically relevant, taking biopsies from suspicious lesions. Teaching medical students, medical doctors in more

advanced levels of education and doctors in primary care medicine about relevant symptoms and the clinical picture of PeIN is important for increasing knowledge on whom and when to investigate further with biopsies and refer to dermatologists or urologists. Even dermatologists and urologists need further training about PeIN and penile cancer and treatments of choice, due to the rarity of PeIN, different clinical appearances and different treatment options. The increasing incidence of PeIN also highlights the importance of starting clinics for patients with PeIN lesions in departments of dermatology and urology. The increasing incidence of PeIN also necessitates further research on treatment outcome with the different potential treatments and on risk of recurrence.

Increased OR for inflammatory skin diseases, balanoposthitis, and having had penile surgical procedures before, highlight the importance of treatment and follow-up of inflammatory skin diseases, and when PeIN is treated, following up the patients according to the national recommendations.

Increased OR for PeIN when taking immunosuppressive drugs and being organ transplanted, points to the significance of examining the skin, including the genital area, in organ-transplanted patients for suspect lesions, with an extra focus in the future on all patients medicating with the relatively new biological treatments for psoriasis and rheumatological diseases.

The increased OR for PeIN in patients with genital warts, as a surrogate marker for HPV prevalence, shown here, supports the importance of informing parents and children about the HPV vaccine in school vaccination programmes and the need for boys to be vaccinated in order to prevent malignant transformation from HR HPV to PeIN and penile cancer.

The finding of a high amount of treatable skin disease (which can potentially lead to PeIN) and clinically not suspected PeIN lesions in excised preputium, underlines the importance of sending circumcised foreskin in symptomatic men for histopathological evaluation, and offering treatment and follow-up when needed.

Despite guidelines there is a local tradition in Sweden of not sending circumcised tissue for histopathological evaluation when no clinically suspicious lesion is present. The results from our study show that this tradition needs to change and information and discussion with the urology society is necessary to change the predominant common practice.

Knowledge of the precise contribution of HPV to penile cancer will help clinicians to recommend and propose HPV vaccination in boys. If HPV can explain only around 40% of the penile cancers and vaccination is included in the school vaccination programme even for boys, then focus needs to be on the other main risk factor, inflammatory skin diseases.

Lee et al. (86) have shown long-term treatment with highly potent steroids reduces symptoms, scarring and cancer development in vulvar LS. Since morphologically it

is the same disease on the penis, the results of long-term treatment are expected to be the same in men with LS. Urologists and dermatologists need to assess and focus on treatment and follow-up of LS and LP to prevent the development of penile cancer. Dermatology departments would benefit from setting up programmes for men with penile skin disease. These programmes could be similar to those for women with vulvar disease.

Gender perspectives in medicine have usually pointed out a negative gender bias for women, for example they receive less advanced investigations and less medical treatment for the same disease compared to men. However, maybe this is one field where men are disadvantaged? To our knowledge, there are seldom dedicated penile clinics in dermatology departments, but these should be promoted to ensure equal treatment of men and women with genital inflammatory diseases.

Shedding light on the viral activity of HPV in penile cancer is crucial to understanding the malignant potential of the HPV virus in penile cancer and thereby developing eventual future therapeutic vaccines and treatments for PeIN and penile cancer.

Future research

PeIN is not well studied, and future research is needed on different aspects, some of which are highlighted below. HPV is well studied in cervical cancer but more rarely in penile cancer, especially regarding how the viral activity leads to penile cancer.

Further studies of the incidence of PeIN and penile cancer are warranted. Will the increasing incidence in PeIN be followed by an increase in invasive cancer? Is the incidence of PeIN going to continue to increase?

The risk of PeIN lesions becoming invasive needs to be addressed in a prospective study. Categorisation of the PeIN lesion according to the new classification into *differentiated* and *undifferentiated* PeIN is important in order to predict and compare the risks of malignant transformation in different PeIN subtypes.

The treatment outcome of PeIN lesions would preferably be studied in an RCT or a study with a prospective design in order to follow PeIN patients to see which treatment has the best effect and which treatment has the highest risk of recurrence. Importantly, the classification of PeIN lesions needs to follow the recent classification into *undifferentiated* and *differentiated*. Do *differentiated* and *undifferentiated* PeIN lesions have different treatment outcomes or different risks of recurrence according to the treatment method used?

Regarding risk factors for PeIN, inflammatory skin diseases such as genital LS and genital LP in men warrant more studies concerning the malignant potential. Prospective studies of treatment of LS in men are needed to be able to verify whether

the malignant progression can be prevented. It would be interesting to calculate relative risk for the malignant progression of LS into PeIN.

Additionally, other potential risk factors for PeIN need to be studied, for example with a focus on the risk of PeIN in patients on systemic biological treatments after several years of treatment. Also smoking, obesity, diabetes, sexual orientation, occupation and contact with chemical substances need to be further investigated to estimate their eventual contribution in PeIN. When investigating BMI and diabetes as potential risk factors, focus needs to be not only on correlations but also on cellular mechanisms and data needs to be adjusted for potential confounders such as socioeconomic factors.

It would also be interesting to see if patients with PeIN have other HPV-related neoplasias, such as AIN and anal cancer. Another interesting framing would be to compare patients with anal cancer and AIN regarding sexual orientation and sexual practice, medication with immunosuppressive medication, organ transplantation and HIV status with HPV status.

The malignant potential of HPV in penile cancer needs further investigation to understand if the virus acts differently to cervical cancer. What is the main cause of HPV DNA integration in penile cancer? Is integration not relevant to the malignant potential in penile cancer? Could a therapeutic vaccine be used to treat HPV-positive PeIN lesions?

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