Örebro Studies in Medicine 358

ÖREBRO UNIVERSITY

DOMINIK GLOMBIK

Penile cancer: Diagnosis, prognosis, and treatment

Author: DOMINIK GLOMBIK
Title: Penile cancer: Diagnosis, prognosis, and treatment

Publisher: Örebro University, 2026
www.oru.se/publikationer

Print: Örebro University, Repro 05/2026
Cover image: The cover image was generated using artificial intelligence (AI).

ISSN: 1652-4063
ISBN: 978-91-7529-784-2 (print)
ISBN: 978-91-7529-785-9 (pdf)

Abstract

The aim of this thesis is to optimise the clinical management and prognostic evaluation of penile cancer (PeCa) by investigating its long-term consequences and treatment-related morbidity, as well as by evaluating current surgical strategies and novel biomarker-based approaches for accurate lymph node staging.

In **Paper I**, nationwide register data demonstrated that patients with PeCa face a two- to threefold increased risk of developing second HPV-associated malignancies in the oral cavity, oropharynx, and anal canal, highlighting the need for improved surveillance and preventive measures. In **Paper II**, population-based analyses demonstrated that morbidity following lymph node dissection remains substantial over time, with significantly elevated risks of infectious complications persisting for more than five years and thromboembolic events for up to three years postoperatively, underscoring the importance of long-term complication awareness. In **Paper III**, an evaluation of a panel of 14 soluble immune checkpoint proteins (sICs) for predicting lymph node metastases (LNM) revealed limited clinical utility due to low sensitivity and modest accuracy. However, four inhibitory sICs (IDO, TIM-3, CD80, and CTLA-4) were significantly elevated in patients with PeCa compared to cancer-free controls, suggesting tumour-induced systemic immunosuppression. In **Paper IV**, dynamic sentinel node biopsy (DSNB) was shown to effectively detect LNM while maintaining favourable morbidity, although a false-negative rate of 14.5% was observed during a median follow-up of 34 months. Complications, predominantly mild to moderate, occurred in 14.8% of groins and were directly associated with higher lymph node yields, emphasizing the critical importance of precise and targeted excision of true sentinel nodes.

Keywords: biomarkers, complications, dynamic sentinel node biopsy, human papillomavirus, immune checkpoint proteins, inguinal lymph node dissection, penile cancer, prognostic factors

To

Andrea, Hanna, and Adam

My greatest achievement is you

Table of Contents

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Glombik, D., Oxelbark, Å., Sundqvist, P., Carlsson, J., Lambe, M., Drevin, L., Davidsson, S., & Kirrander, P. (2021). Risk of second HPV-associated cancers in men with penile cancer. *Acta Oncologica (Stockholm, Sweden)*, *60*(5), 667–671.

II. Glombik, D., Davidsson, S., Sandin, F., Lambe, M., Carlsson, J., Sundqvist, P., & Kirrander, P. (2023). Penile cancer: long-term infectious and thromboembolic complications following lymph node dissection—a population-based study (Sweden). *Acta Oncologica (Stockholm, Sweden)*, *62*(5), 458–464.

III. Glombik, D., Carlsson, J., Kirrander, P., & Davidsson, S. (2026). Soluble immune checkpoint proteins as predictive biomarkers for lymph node metastases in penile cancer. *Frontiers in Immunology*, *17*, 1754254.

IV. Glombik, D., Davidsson, S., Carlsson, J., & Kirrander, P. Dynamic sentinel node biopsy in penile cancer: Sensitivity and complication rate in a tertiary national referral centre. *In manuscript.*

Reprints were made with permission from the respective publishers.

List of abbreviations

ASCO	American Society of Clinical Oncology
AUC	Area under the curve
BMI	Body mass index
CI	Confidence interval
cN	Clinical N stage
cT	Clinical T stage
CT	Computed tomography
DSNB	Dynamic sentinel node biopsy
EAU	European Association of Urology
FDG-PET/CT	Fluorodeoxyglucose positron emission tomography/ computed tomography
FN	False negative
FNAC	Fine-needle aspiration cytology
G	Grade
HR	Hazard ratio
HPV	Human papillomavirus
ILND	Inguinal lymph node dissection
IC	Immune checkpoint
IQR	Interquartile range
LN	Lymph node
NPECR	National Penile Cancer Register
NPR	National Patient Register
OR	Odds ratio

PeIN	Penile intraepithelial neoplasia (Carcinoma in situ)
PenBUS	Penile Blood and Urine Study
PenCBaSe	Penile Cancer Data Base Sweden
PLND	Pelvic lymph node dissection
pN	Pathologic N stage
pT	Pathologic T stage
SCC	Squamous cell carcinoma
SCR	Swedish Cancer Register
sIC	Soluble immune checkpoint protein
SN	Sentinel node
SPECT/CT	Single-Photon Emission Computed Tomography/ Computed Tomography
Tis	Carcinoma in situ
TME	Tumour microenvironment
TNM	Tumour, nodes, metastases
US	Ultrasound

Introduction

Penile cancer is a rare malignancy with a global incidence of 0.8 per 100,000 men (1). Although the incidence remains low in most Western countries, higher incidence rates have been reported in parts of South America, Africa, and Asia (2). In Sweden, the incidence has increased over the past two decades and now exceeds 2 per 100,000 men, corresponding to approximately 140 new cases annually (Figure 1) (3). Despite a relatively high mean age at diagnosis of 72 years, the disease also affects younger men, with approximately one quarter of patients diagnosed before the age of 60 (4, 5).

Squamous cell carcinoma (SCC) constitutes more than 95% of cases and most commonly arises on the glans penis or inner prepuce (6). Although uncommon, the treatment of penile cancer is associated with substantial morbidity, limiting overall quality of life with the greatest impact on voiding and sexual functions (7, 8). Prognosis is strongly influenced by the presence and extent of inguinal lymph node metastases. Patients with localised disease have the most favourable outcomes, with a five-year cancer-specific survival of up to 96%, which decreases rapidly to only 37% in patients with advanced nodal disease (N3) (9).

Early diagnosis, accurate staging enabling the detection of micrometastases, and timely treatment are essential to improve survival and guarantee the best possible quality of life.

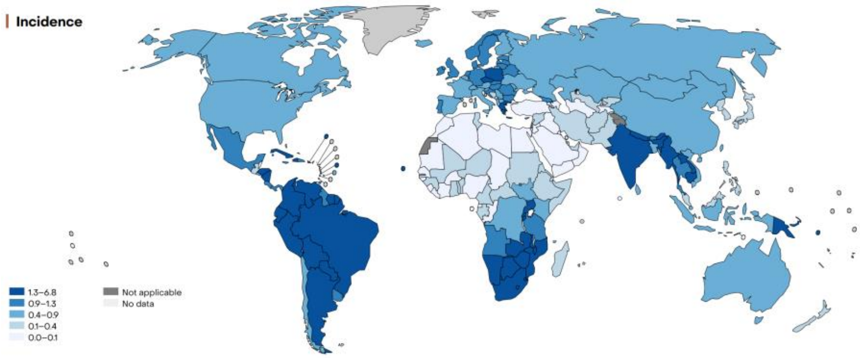

Figure 1. Age-standardised incidence rates. Penile cancer, males, all ages. Data are from the Global Cancer Observatory (10).

Aetiology and pathogenesis

The aetiology of penile cancer is multifactorial. Established risk factors include human papillomavirus (HPV) infection, chronic inflammatory conditions, phimosis, and tobacco smoking (2). Conversely, circumcision performed early in life and HPV vaccination are considered protective. The protective effect of early circumcision is likely achieved through the prevention of phimosis, a known risk factor for chronic inflammation (11). HPV vaccination reduces the incidence and persistence of high-risk HPV infections, subsequently reducing the burden of HPV-associated cancers in the population (12, 13).

Currently, two independent pathways are considered in penile carcinogenesis: an HPV-associated pathway and an HPV-independent pathway driven predominantly by chronic inflammation (14, 15).

In addition, approximately 30% of penile cancer precursor lesions, i.e., penile intraepithelial neoplasia (PeIN), progress to invasive cancer if left untreated (16). However, when identified and successfully treated, progression rates decrease markedly to only 2–7% (17, 18).

HPV-associated pathway of penile carcinogenesis

HPV is the most common oncogenic virus worldwide and is estimated to contribute to approximately 5% of the global cancer burden

(19). It is the most common sexually transmitted virus, particularly among sexually active young adults. Nearly all sexually active individuals are infected at some point during life. In most cases, the infection is transient and cleared spontaneously by the immune system within 1–2 years (20). Persistent infection with high-risk HPV types, however, may induce the malignant transformation of infected epithelial cells. Among the oncogenic HPV types, HPV16 and HPV18 are the most clinically important and the most frequently detected in HPV-associated cancers. In penile cancer, HPV16 is also the most common high-risk genotype, responsible for almost 70% of HPV-positive cases, followed by the less prevalent HPV18, HPV31, HPV33, and HPV45 genotypes (21, 22).

HPV infections are associated with several cancer types such as anogenital (penile, anal canal), head and neck (oral cavity, oropharyngeal), and gynaecological (cervical, vaginal, vulvar) malignancies (23). In cervical cancer, HPV is detected in virtually all tumours and constitutes the dominant aetiological factor. In penile cancer, HPV DNA has been identified in up to 50% of cases, with the highest prevalence observed in basaloid and warty subtypes. HPV positivity is also more commonly seen in PeIN than in invasive penile cancer, supporting its role in early malignant transformation (14, 24, 25).

HPV-associated tumours appear to represent a biologically distinct subgroup characterised by specific histology, a lower risk of metastases, and an improved response to treatment (26, 27). Several studies have reported a better prognosis for patients with HPV-associated penile cancer, with improved cancer-specific survival compared with HPV-negative penile cancer (28). Different immune escape mechanisms utilised by HPV-negative tumour cells may contribute to the worse prognosis observed in these patients (29).

The implications of HPV infection may extend beyond the primary penile tumour. Persistent infection and possible cross-infection across multiple anatomic sites raise the possibility of subsequent HPV-associated cancers. This association is well established in women with cervical cancer, whereas the corresponding risk in men with penile cancer remains less clearly defined (30, 31). A better understanding of this issue may have implications for surveillance, patient counselling, and preventive strategies, since most precancerous and early-stage cancers caused by HPV can be treated successfully.

HPV-independent pathway of penile carcinogenesis

In addition to HPV infection, chronic inflammatory conditions represent an important pathway in penile carcinogenesis. Disorders such as lichen sclerosus, lichen planus, and balanoposthitis are frequently implicated (32). While inflammation is a protective host response to infection or tissue injury, unresolved inflammation may become chronic and lead to further tissue damage.

Chronic inflammation promotes a tissue microenvironment that favours malignant transformation (33). Persistent inflammatory signalling may lead to oxidative stress, DNA damage, aberrant tissue remodelling, increased cellular proliferation, and impaired apoptosis. Over time, this may result in genomic instability and the accumulation of mutations that favour the emergence of tumour cell clones with growth and survival advantages. Non-resolving inflammation is therefore recognised as an enabling characteristic of carcinogenesis (34). It is estimated that approximately 25% of all cancers are caused by infections and chronic inflammation (35).

Impact of the immune system and immune checkpoint proteins

The immune system plays a critical role in the recognition and elimination of malignant cells through the anti-tumour immune response. This process involves both the innate and adaptive immune systems working together to detect and eliminate transformed cells. Tumour-associated antigens released from cancer cells are captured and processed by antigen-presenting cells, predominantly dendritic cells, which migrate to the lymphoid organs and present these antigens to naïve T cells. This interaction leads to the activation and clonal expansion of tumour-specific $CD8^{+}$ cytotoxic T cells, which are key mediators of anti-tumour immunity. The entire process is illustrated by the cancer-immunity cycle (Figure 2) (36).

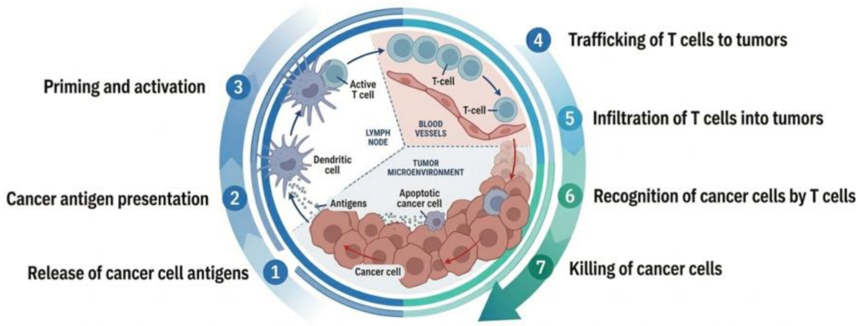

Figure 2. The cancer-immunity cycle illustrating the seven steps of anti-tumour immune response. AI-generated image.

During cancer development, tumour cells acquire mechanisms that enable them to escape immune recognition and elimination. One important mechanism by which tumours evade anti-tumour immunity is through the modulation of immune checkpoint pathways that regulate T-cell activation and immune tolerance (37).

Immune checkpoint proteins can be broadly classified into stimulatory and inhibitory molecules. Stimulatory checkpoint proteins enhance T-cell activation, proliferation, and survival. Examples of such molecules include glucocorticoid-induced tumour necrosis factor receptor-related protein (GITR) as well as cluster of differentiation 27 (CD27), CD28, and CD137. These receptors provide co-stimulatory signals that support effective immune responses against tumour cells (38).

In contrast, inhibitory checkpoint proteins suppress T-cell activation and help maintain immune homeostasis. Key inhibitory checkpoint molecules include B- and T-lymphocyte attenuator (BTLA), indoleamine 2,3-dioxygenase (IDO), lymphocyte-activation gene 3 (LAG-3), herpes virus entry mediator (HVEM), programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), PD-L2, T-cell immunoglobulin and mucin domain 3 (TIM-3), CD80, and CD152 (CTLA-4). Activation of these inhibitory pathways can suppress T-cell proliferation and cytotoxic activity, ultimately weakening anti-tumour immunity (Figure 3) (39).

Tumours can exploit this immune regulatory system to escape immune-mediated destruction. A primary strategy involves the up-regulation of inhibitory immune checkpoint proteins. For example, tumour cells often overexpress PD-L1 and PD-L2, which bind to PD-1 on $CD8^+$ T cells, leading to reduced proliferation and cytotoxic activity. At the same time, tumours may down-regulate stimulatory immune checkpoint proteins, required for full T-cell activation. Therefore, clinical outcomes are likely determined by the balance between stimulatory and inhibitory immune checkpoint signalling within the tumour microenvironment.

Most previous research investigating the impact of immune checkpoint proteins in cancer development has been based on tissue-level analyses. These studies primarily focus on the expression pattern within the tumour and have provided valuable insights into tumour–immune interactions and correlations between immune checkpoint expression and clinical outcomes. Increased expression of inhibitory checkpoint proteins in tumour tissue has been associated with adverse clinicopathological features and poorer outcomes in several malignancies, including melanoma, urinary bladder, kidney, pancreatic, and lung cancers. In penile cancer, increased expression of PD-L1 has been associated with inguinal lymph node metastases and more aggressive disease (40-42). In addition, high expression of several inhibitory immune checkpoint proteins was observed in the primary tumour in patients with advanced penile cancer (29, 43). This indicates that tumour expression of immune checkpoint proteins may possess value as prognostic biomarkers in penile cancer.

Tissue-based evaluations have traditionally been used to predict prognosis in penile cancer patients. While informative, these approaches are relatively invasive, so less invasive alternatives, such as blood-based analyses, are desirable. More recently, soluble forms of immune checkpoint proteins have emerged as a topic of interest. Compared with their membrane-bound counterparts within tumour tissue, these soluble proteins can be detected in body fluids, including blood (plasma and serum). Importantly, many of these soluble molecules retain biological activity and can interact with their corresponding receptors or ligands, potentially modulating systemic anti-tumour immunity. This capability suggests that soluble immune checkpoint proteins could reflect not only the local tumour microenvironment but also the broader immune status of the patient (44).

The use of liquid biopsy to detect soluble immune checkpoint proteins offers several advantages. It is minimally invasive compared with tissue biopsy, allows repeated sampling over time, and can provide dynamic information on tumour–immune interactions, treatment response, and emerging tumour recurrence. Such an approach may prove valuable for early detection, prognostication, and treatment monitoring. However, in penile cancer, studies examining soluble immune checkpoint proteins remain limited, and their prognostic value is still largely unexplored. Expanding research in this area could provide a tool for the personalised management of penile cancer patients.

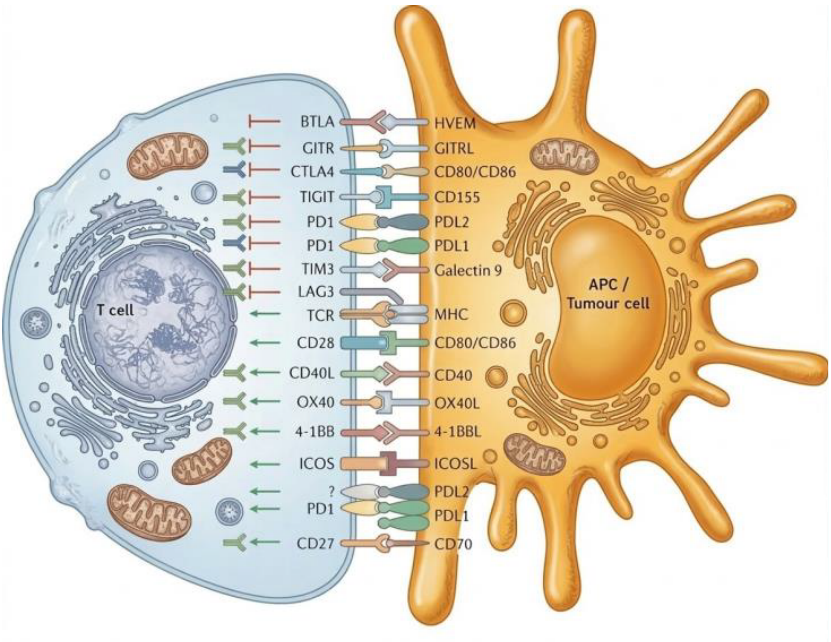

Figure 3. Receptor–ligand interactions of different immune checkpoint proteins on an antigen presenting cell (APC)/tumour cell and a T cell. Inhibitory checkpoints are marked with red square arrows and stimulatory checkpoints with green arrows. AI-generated image.

Staging

Penile cancer can often be treated successfully if it is localised or when metastases are discovered early before spreading beyond the inguinal lymph nodes. Initial diagnosis is based on the physical examination of the penis and palpation of regional lymph nodes, followed by biopsy if the diagnosis is uncertain. Imaging is reserved for patients with a suspicion of invasive cancer (≥cT1) or if pathological inguinal lymph nodes are suspected (cN+) or confirmed (pN+) (6).

The stages of penile tumours are determined according to the Union for International Cancer Control (UICC) TNM Classification system, which was updated to its 9th edition in 2025 (Table 1, Figure 4) (45). The 9th edition contains no changes in penile cancer staging compared with the 8th edition of the UICC TNM Classification system which was introduced in 2017 (46).

Penile cancer follows a stepwise dissemination pattern, initially affecting the inguinal lymph nodes, where the sentinel nodes are located, followed by the pelvic lymph nodes prior to systemic dissemination (47, 48). The presence and extent of lymph node metastases is the most important prognostic factor and fundamentally determines both the treatment strategy and survival (28, 49). Most patients with penile cancer are diagnosed with localised disease, but approximately 14% of patients will present with clinically suspicious inguinal lymph nodes (cN+). Nevertheless, approximately 20–25% of patients without clinically suspicious inguinal lymph nodes (cN0) harbour occult metastases, as current imaging lacks sensitivity to detect micrometastases (≤2mm), and no validated predictive biomarkers are yet available (49, 50). Additional surgical staging is therefore warranted.

Traditionally, most patients have been referred to diagnostic inguinal lymph node dissection (ILND), a procedure associated with morbidity rates of 35% to 70% (51-53). Hence, a major challenge is to correctly select patients for therapeutic lymph node dissection. If all patients undergo diagnostic ILND, approximately 80% will be overtreated, with a risk of severe adverse effects on quality of life and without any survival benefits. However, in patients with lymph node metastases, delayed surgery is associated with worse outcomes (49).

To address the shortcomings of diagnostic ILND, dynamic sentinel node biopsy (DSNB) was introduced as a minimally invasive approach intended to selectively identify the first draining lymph node(s) from the primary tumour (47, 54). This represented a major step forward in correctly selecting patients who would benefit from lymph node dissection while avoiding unnecessary untoward consequences in the majority of clinically node-negative patients. Therapeutic lymph node dissection, due to its considerable morbidity, could be reserved only for patients with confirmed lymph node metastases (pN+).

Currently, clinically node-negative patients (cN0) are stratified into risk groups based on histopathological features of the primary tumour as these are central to estimating the risk of lymph node metastases (Table 2) (50, 55).

For primary tumours with a low risk of lymph node metastases, surveillance with physical examination of the regional lymph nodes is recommended. For intermediate- and high-risk tumours (≥pT1G2) without evidence of regional or distant metastases on computed tomography (CT) and ultrasound (US) with the option of fine-needle aspiration cytology (FNAC) of any suspicious inguinal lymph nodes, surgical nodal staging with DSNB is recommended (6, 56).

Table 1. TNM classification of penile tumours (9th edition).

	Primary tumour (T)
Clinical/pathologic stage definition	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	PeIN (Carcinoma in situ)
Ta	Non-invasive localised squamous cell carcinoma, including verrucous carcinoma
T1	Tumour invades subepithelial connective tissue
T1a	Tumour invades subepithelial connective tissue without lymphovascular invasion or perineural invasion and is not poorly differentiated (i.e. not grade 3)
T1b	Tumour invades subepithelial connective tissue with lymphovascular invasion or perineural invasion, or is poorly differentiated
T2	Tumour invades corpus spongiosum with or without urethral invasion
T3	Tumour invades corpus cavernosum with or without urethral invasion
T4	Tumour invades other adjacent structures
	Regional lymph nodes (N)
Clinical stage definition	
cNX	Regional lymph nodes cannot be assessed
cN0	No palpable or visibly enlarged inguinal lymph nodes
cN1	Palpable mobile unilateral inguinal lymph node
cN2	Palpable mobile multiple or bilateral inguinal lymph nodes
cN3	Fixed inguinal nodal mass or pelvic lymphadenopathy, unilateral or bilateral
Pathologic stage definition	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in 1 or 2 unilateral inguinal lymph nodes
pN2	Metastases in more than 2 unilateral inguinal lymph nodes or in bilateral inguinal lymph nodes
pN3	Metastasis in pelvic lymph node(s), unilateral or bilateral, or extranodal extension of regional lymph node metastasis
	Distant metastasis (M)
M0	No distant metastasis
M1	Distant metastasis, including lymph node metastasis outside the regional lymph nodes (inguinal and pelvic nodes), as well as visceral or bone metastasis
	Histopathological grading (G)
GX	Grade of differentiation cannot be assessed
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated or undifferentiated

PeIN = Penile Intraepithelial Neoplasia

Table 2. Risk categories for regional lymph node metastases based on pathologic stage and grade.

Pathologic stage	Risk group	Risk of lymph node metastases
pTa, pT1aG1	Low risk	<6%
pT1aG2	Intermediate risk	6–8%
≥ pT1bG2	High risk	22–30%

Dynamic sentinel node biopsy (DSNB)

The European Association of Urology (EAU) – American Society of Clinical Oncology (ASCO) guidelines as well as the Swedish national guidelines consider DSNB the gold standard staging procedure for clinically node-negative patients (cN0) with intermediate- and high-risk primary tumours (≥T1G2) (6, 56). The sensitivity of this minimally invasive staging procedure is reportedly up to 95% when performed at high-volume centres (57). Compared with ILND, DSNB is associated with fewer and less severe complications, and it confers a better prognosis than surveillance alone (58-60).

The DSNB protocol includes preoperative US of both groins with the option of FNAC of any suspicious inguinal lymph nodes and lymphoscintigraphy using 99mTc-nanocolloid. Dynamic lymphoscintigraphy is acquired for the first 10 min after tracer administration, followed by early (15 minutes) and late (90 minutes) static planar images and SPECT/CT (90–120 minutes) for the precise anatomical localisation of sentinel nodes. Intraoperatively, patent blue dye is injected intradermally at the penile base to facilitate the optical identification of sentinel nodes in the surgical field. Sentinel nodes are then identified using a handheld gamma probe in combination with visual inspection and palpation (48, 61, 62).

US with FNAC on indication has become an integral part of the DSNB algorithm to avoid false-negative (FN) cases of nodal metastasis caused by tumour blockage. Furthermore, in the case of a positive US with FNAC, the ipsilateral therapeutic ILND can be performed at an earlier stage, reducing the need for further DSNB by 10–13% (63, 64).

Management of primary tumour

The main objective in the management of primary tumours is complete excision with negative surgical margins. Concurrently, maximising the preservation of penile length and glans epithelium, when oncologically safe, is of high priority to maintain good voiding ability, sexual function, and cosmetic appearance (65-67). The choice between an organ-sparing strategy and penectomy (partial or total) is mainly dictated by the histopathological stage and grade, tumour size, and anatomical location. However, the patient's body constitution or fear of local recurrence may lead them to opt for more radical treatment.

Organ-sparing treatment

For non-invasive disease (PeIN, Ta) and early-stage invasive tumours (T1, T2), organ-sparing approaches are considered the gold standard (Figure 4). These procedures include circumcision, local excision, glans resurfacing, and glansectomy with or without neoglans reconstruction using a split-thickness skin graft. While organ-sparing techniques yield excellent functional and cosmetic outcomes, they are associated with a higher risk of local recurrence compared with more radical penectomy. However, no negative impact on overall or metastasis-free survival has been observed (65, 68).

Topical chemotherapy with 5-fluorouracil or topical immunotherapy with imiquimod are viable, well-established options for patients with PeIN lesions who prefer non-surgical treatment (6, 56). Surgical intervention, such as glans resurfacing or local excision, may still be indicated for disease refractory to topical agents. Careful patient selection and rigorous, long-term follow-up are mandatory when opting for organ-sparing treatment (17, 69).

Non-organ-sparing treatment

In locally advanced penile cancer (proximally located T2, T3, and T4) or when organ-sparing surgery is not anatomically feasible, non-organ-sparing treatments are required (Figure 4). These include partial penectomy and total penectomy with perineal urethrostomy. The choice between a partial and total penectomy largely depends on the ability to preserve a functional penile stump for voiding while securing negative surgical margins. Selected patients undergoing partial penectomy can be offered neoglans reconstruction with the use of a split-thickness skin graft. Occasionally, a patient's choice of non-organ-sparing surgery driven by a desire for greater oncological certainty with lower risk of local recurrence needs to be considered.

While surgical resection with wide margins provides the most reliable local oncological control, the traditional recommendation of a broad surgical margin is no longer considered mandatory. Contemporary evidence demonstrates that much narrower margins are oncologically safe, provided that complete excision with histologically negative margins is achieved (70-72). Consequently, organ-sparing techniques should be prioritised whenever clinically and anatomically feasible, as penectomy is associated with a profound impact on patients' quality of life, body image, and sexual function.

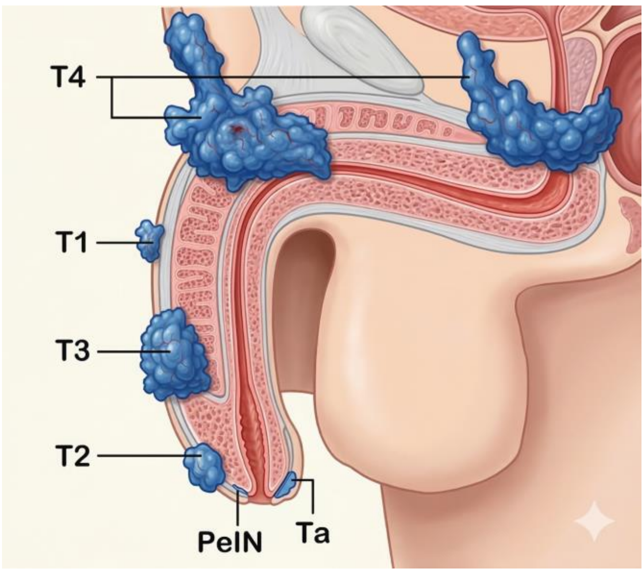

Figure 4. T-stages of primary tumour according to the 9th edition of the TNM Classification system. AI-generated image.

Management of lymph node metastases

For patients with confirmed regional metastasis, timely lymph node dissection is of paramount importance. Early surgical resection is critical to prevent further lymphatic spread or systemic dissemination and remains the key to achieving optimal therapeutic outcomes and long-term survival. Cancer-specific survival decreases drastically from 96% in pN0 to 80%, 66%, and 37% in pN1, pN2, and pN3 stages, respectively (9). The extent of lymph node dissection is guided by the burden of nodal involvement owing to a predictable pattern of metastatic spread.

Inguinal lymph node dissection (ILND)

Historically, ILND has served as the cornerstone of both the staging and treatment of regional lymph nodes in penile cancer. For clinically node-negative patients (cN0) with high-risk primary tumours, a modified ILND can be utilised as a staging procedure to identify occult metastases and reduce the severe morbidity associated with radical ILND (73). Despite the limited anatomical extent of the modified ILND compared with the radical ILND, the complication rates remain substantial. Consequently, DSNB has largely superseded modified ILND and is now the recommended, minimally invasive staging procedure for cN0 patients (Figure 5).

Currently, a radical ILND is strictly indicated when metastatic disease is confirmed by US with FNAC in patients with clinically suspicious inguinal lymph nodes or by a positive DSNB (6, 56). In the therapeutic setting, the procedure aims for the timely, radical excision of the inguinal lymph nodes as cure can be achieved only before metastatic spread beyond regional lymph nodes.

Fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) has demonstrated high sensitivity (87%) and specificity (88%) in detecting pelvic metastases and systemic dissemination (74). Consequently, the Swedish national guidelines have been updated to recommend FDG-PET/CT for all patients with confirmed lymph node metastases (pN+) prior to a final treatment decision (56). The EAU–ASCO guidelines strongly recommend FDG-

PET/CT for staging the pelvis and detecting distant metastases in clinically node-positive patients (cN+) (6).

Pelvic lymph node dissection (PLND)

The total number of ipsilateral inguinal lymph node metastases and the presence of extranodal extension (ENE) are two established predictors of pelvic nodal involvement (75, 76). Therefore, the Swedish national guidelines recommend an ipsilateral PLND when two or more pathological inguinal lymph nodes, or ENE (pN3) are detected (56). This contrasts with the EAU–ASCO guidelines, which recommend ipsilateral PLND only in cases of ENE or when three or more ipsilateral inguinal lymph node metastases are present (6). Cross-over metastases from the inguinal lymph nodes to the contralateral pelvic lymph nodes are extremely rare and supported by only limited evidence (77).

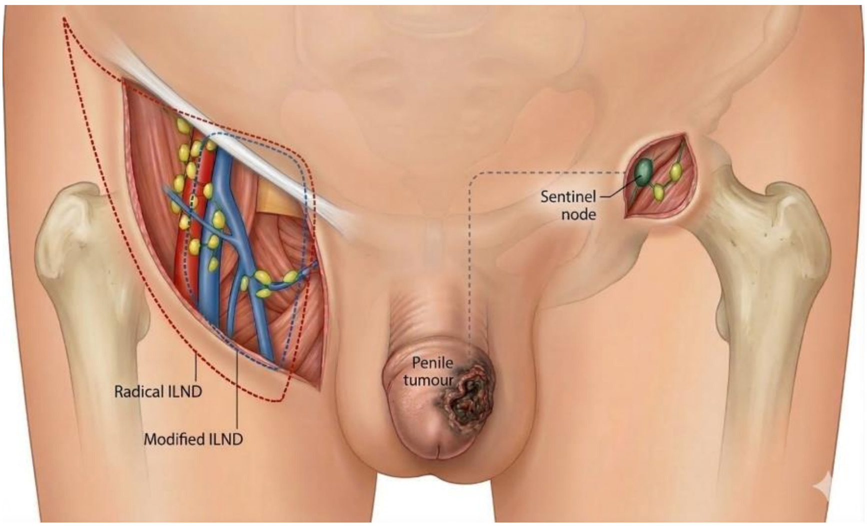

Figure 5. Metastatic dissemination of penile cancer. The surgical template of modified ILND is outlined by a blue dashed line and radical ILND by a red dashed line. AI-generated image.

Lymph node dissection complications

Despite its curative intent, lymph node dissection is associated with significant morbidity, with reported complication rates ranging from 35% to 70% (51, 52, 78).

Early postoperative complications and risk factors

The majority of early complications associated with ILND are wound related. The most frequently reported adverse events include wound infection, dehiscence, skin-flap necrosis, lymphocele, and seroma formation (51, 52, 79). While limited data are available regarding PLND in penile cancer, complications generally follow a similar profile of wound-related events, alongside an elevated risk of thromboembolism (75).

The risk of developing postoperative complications is influenced by several patient-specific and surgical factors, such as high body mass index (BMI), low overall physical status, prolonged operating time, bilateral dissection, and a higher number of excised lymph nodes (80). Furthermore, the presence of bulky nodal disease (cN3) strongly correlates with increased surgical complexity and a higher risk of adverse events (81).

Late-occurring complications

Late-occurring complications can be equally debilitating. Lymphoedema of the lower extremities and genitalia is the most prevalent chronic condition, arising from impaired lymphatic drainage and severely impacting daily functioning (81). Moreover, patients with lymphoedema are highly susceptible to recurrent skin and soft tissue infections, such as erysipelas, cellulitis, and lymphangitis (79). Additionally, due to the nature of the extensive surgery and underlying malignancy, these patients remain at an increased risk of deep vein thrombosis (DVT) and pulmonary embolism (PE) for several months postoperatively (79, 82, 83).

Preventive measures

To mitigate the high morbidity, several preventive measures are essential. Intraoperatively, antibiotic prophylaxis and meticulous tissue handling are crucial. Postoperatively, pharmacologic DVT prophylaxis and early mobilisation are essential. Furthermore, compression stockings are routinely used to minimise the development of both lymphoedema and thromboembolism (84, 85).

Chemotherapy

For patients with advanced regional disease, and particularly in pN3 disease, surgery alone often fails to achieve long-term survival (86, 87). In these cases, a multimodal approach integrating systemic chemotherapy is essential to downstage the tumour burden, eradicate early micrometastases, and facilitate curative surgery.

According to the Swedish national guidelines, chemotherapy is indicated in eligible patients presenting with two or more unilateral lymph node metastases, ENE, or a lymph node metastasis measuring ≥3 cm. Patients requiring PLND qualify by definition for neoadjuvant chemotherapy. The recommended regimen consists of four cycles of paclitaxel, ifosfamide, and cisplatin (TIP) (56).

Studies evaluating perioperative oncological therapy in advanced penile cancer are scarce, often retrospective in nature, and limited by small cohorts. Nevertheless, the existing evidence strongly supports its use. A recent nationwide Swedish study demonstrated a 37% lower risk of disease-specific mortality among patients receiving perioperative oncological treatment compared to untreated eligible patients (88). Furthermore, patients who achieve an objective response to chemotherapy administered with curative intent show significantly improved survival outcomes, with reported reductions in mortality risk of up to 75% compared with non-responders (89).

Surgical innovations and centralisation of care

To reduce wound-related morbidity, minimally invasive techniques such as robot-assisted video endoscopic inguinal lymphadenectomy (RA-VEIL) have been introduced. Compared with the traditional open approach, RA-VEIL is associated with significantly lower rates of wound-related complications. However, these benefits come at the expense of longer operating times and a steep learning curve (90).

Given the multimodal nature of DSNB and the substantial complication profile of radical ILND, there is a strong rationale for centralising the management of this rare cancer. Highly specialised, high-volume centres with multidisciplinary expertise and well-coordinated perioperative pathways are better equipped to ensure optimal oncological and functional outcomes without unwarranted variations in care (91-94).

In Sweden, this rationale has driven a major reorganisation of penile cancer care over the past decade. To ensure equitable and high-quality treatment nationwide, a weekly national multidisciplinary team (MDT) conference was established in 2013, mandating the review of all newly diagnosed cases and relapses. Subsequently, in 2015, curative penile cancer surgery was centralised to two national tertiary referral centres: Örebro University Hospital and Skåne University Hospital in Malmö. This centralisation aimed to improve adherence to clinical guidelines, reduce surgical morbidity, and facilitate clinical research. In 2017, a standardised cancer patient pathway was introduced to streamline the diagnostic process, reduce waiting times, and guarantee equal access to optimal care regardless of the patient's place of residence (56, 88).

AIMS

The overall aim of this thesis is to improve the clinical management and outcomes of patients with penile cancer. This is pursued by evaluating the risk of second HPV-associated malignancies, assessing the risks of treatment-related morbidity, exploring novel biomarkers of nodal dissemination, and optimising surgical staging. The specific aims of the individual papers are listed below.

Paper I

To investigate whether patients diagnosed with penile cancer are at an increased risk of developing a second HPV-associated cancer, specifically oral cavity, oropharynx, or anal cancers.

Paper II

To evaluate the incidence and risk of infections and thromboembolic complications following inguinal and pelvic lymph node dissection in patients with penile cancer, and to identify clinical and pathological predictors of these adverse events.

Paper III

To explore the clinical utility of a panel of 14 soluble immune checkpoint proteins as predictive biomarkers for detecting lymph node metastases in penile cancer, and to assess their potential as systemic indicators of tumour-induced immunomodulation by comparing their plasma concentrations with those of cancer-free controls.

Paper IV

To determine the sensitivity and false-negative rate of dynamic sentinel node biopsy for detecting occult inguinal lymph node metastases, and to assess the incidence and predictors of postoperative complications within a centralised, tertiary referral setting.

Patients and methods

This thesis is based on four complementary studies utilising both retrospective and prospective designs to address different aspects of penile cancer. **Papers I** and **II** are large, retrospective register studies utilising data from the Penile Cancer Data Base Sweden (PenCBaSe), a high-quality, population-based research database generated by record linkages between the National Penile Cancer Register (NPECR) and six other national healthcare and sociodemographic registries. **Paper I** investigates the incidence and risk of second HPV-associated cancers among patients diagnosed with penile cancer. **Paper II** evaluates the long-term risk of infectious and thromboembolic complications in penile cancer patients undergoing lymph node dissection. **Paper III** is a translational biomarker study assessing the predictive value of soluble immune checkpoint proteins in plasma from penile cancer patients and cancer-free controls. Finally, **Paper IV** is a retrospective clinical cohort study evaluating the sensitivity and complication risk of dynamic sentinel node biopsy (DSNB) performed at a national tertiary referral centre.

Papers I and II: Population-based register studies

Study design and population

Papers I and II are retrospective, population-based cohort studies utilising the comprehensive coverage of the Swedish national registries. The population in **Paper I** comprised all patients with a penile cancer diagnosis registered in the NPECR between 2000 and 2012. The study aimed to investigate the risk of developing a second HPV-associated malignancy. **Paper II** focused on a surgical sub-cohort consisting of all patients undergoing ILND, with or without PLND, to evaluate long-term morbidity with a specific focus on infectious and thromboembolic complications (Figure 6).

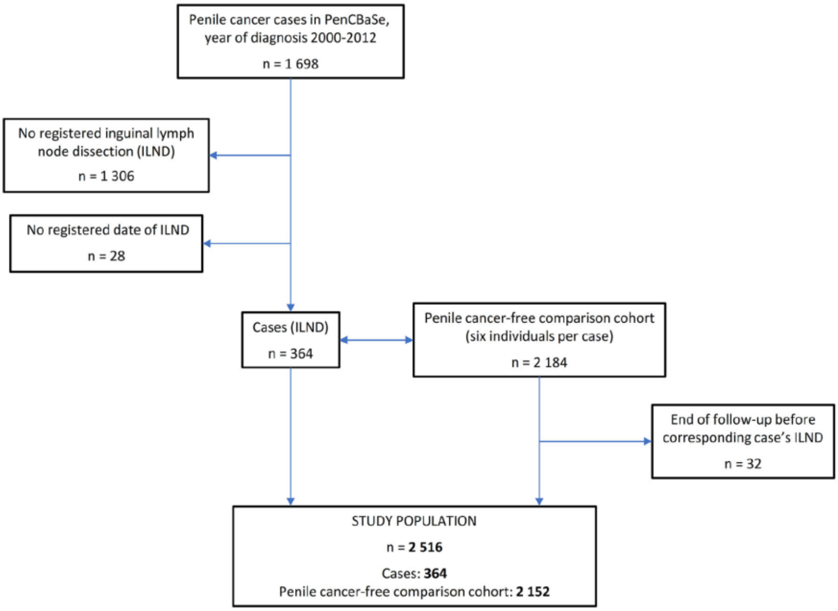

Figure 6. Flowchart illustrating the number of penile cancer patients in the PenCBaSe database together with the number of eligible penile cancer patients (cases) who underwent inguinal lymph node dissection (ILND) and their matched penile cancer-free controls.

Data collection and registries

PenCBaSe was generated by utilising the unique Swedish personal identity number to accurately cross-link the records from the NPECR with the National Patient Register, the Swedish Cancer Register, the Cause of Death Register, the Register of the Total Population, the Swedish Prescribed Drug Register, and the Longitudinal Integration Database for Health Insurance and Labour Market Studies (LISA). Overall, 1698 penile cancer patients diagnosed between 2000 and 2012 were included in PenCBaSe, along with six penile cancer-free controls for each patient, matched by sex, age, and county of residence.

The NPECR was initiated in 2000 to monitor and improve the quality of penile cancer care in Sweden. To date, more than 4000 patients with penile cancer have been reported to the NPECR, representing, to our knowledge, the largest population-based quality register for penile cancer in the world. The NPECR contains information on date of diagnosis, county of residence, tumour characteristics according to the TNM classification, location and size of the tumour, primary treatment, lymph node management, and follow-up data. However, HPV status is not routinely registered. Data quality in the NPECR is generally high with respect to completeness, timeliness, comparability, and validity. Its completeness was 95% between 2000 and 2023 when compared with the Swedish Cancer Register (5, 95).

The Swedish Cancer Register was founded in 1958 and contains data on all new cases of cancer, as reporting by all healthcare providers is mandated by law (96).

The National Patient Register was founded in 1964; however, complete national coverage was achieved for inpatient hospital care in 1987 and for outpatient care provided by both private and public caregivers in 2001. Primary care visits are not covered by the register (97).

The Swedish Prescribed Drug Register was founded in 2005 and holds information on all prescribed drugs dispensed by pharmacies, excluding drugs administered within hospitals. All details regarding Anatomical Therapeutic Chemical (ATC) codes, drug names, pack sizes, strengths, and dates of prescription and dispensation are included (98).

For **Paper I**, linkage with the Swedish Cancer Register enabled the identification of primary malignancies with a known HPV association, specifically, cancers of the oral cavity, oropharynx, and anal canal, in men with and without penile cancer. In addition, incidence rates in the general male population were estimated for these HPV-associated malignancies based on information provided by the National Board of Health and Welfare (99).

For **Paper II**, linkage with the National Patient Register allowed for the capture of specific International Classification of Diseases (ICD) codes associated with infectious complications (e.g., infections of the lower limbs, groins, genitalia, and trunk, as well as various septic conditions) and venous thromboembolic events. Additionally, the prescription of anticoagulants, assessed via specific ATC codes through the Prescribed Drug Register, was used as a surrogate marker of thromboembolic events occurring >30 days postoperatively.

Statistical analysis

In **Paper I**, Cox proportional hazards regression models were used to calculate hazard ratios (HRs) to examine the risk of HPV-associated cancers in patients with penile cancer compared with penile cancer-free controls. Furthermore, standardised incidence ratios (SIRs) were calculated to quantify the relative risk of these second malignancies. The observed number of second HPV-associated cancers in the study cohort was compared with the expected number within the general, age- and calendar period-matched Swedish male population.

In **Paper II**, the net probability of experiencing an adverse event of interest over time was estimated using the Kaplan–Meier method, with individuals censored at the time of death, emigration, or end of follow-up (31 December 2012). Subsequently, univariable and multivariable Cox proportional hazards regression models were applied to assess the effects of specific clinical, pathological, and sociodemographic predictors on the risk of experiencing these adverse events. The evaluated predictors included age, calendar period, comorbidity, marital status, education, cT and cN stage, tumour grade, and type of lymph node dissection (ILND with or without PLND). The results were presented as HRs with 95% confidence intervals (CIs).

Paper III: Translational biomarker study

Study design and population

Paper III is a cross-sectional study with the primary objective of exploring the clinical utility of a panel of 14 soluble immune checkpoint proteins as predictive biomarkers of lymph node metastases. The study population consisted of patients prospectively enrolled in the Penile Blood and Urine Study (PenBUS). The PenBUS cohort comprises both men referred to Örebro University Hospital between 2019 and 2024 for suspected penile cancer, and a control group of cancer-free patients who underwent transurethral resection of the prostate for benign prostatic hyperplasia between 2021 and 2024. All patients with concurrent malignancies, autoimmune disorders, or ongoing immunomodulatory therapy were excluded. After applying the inclusion criteria, the final cohort comprised 284 penile cancer patients and 45 controls. To validate our results, the penile cancer cohort was further stratified based on the date of inclusion in a training set (n = 202; January 2019–April 2022) and a test set (n = 82; May 2022–July 2024) (Figure 7).

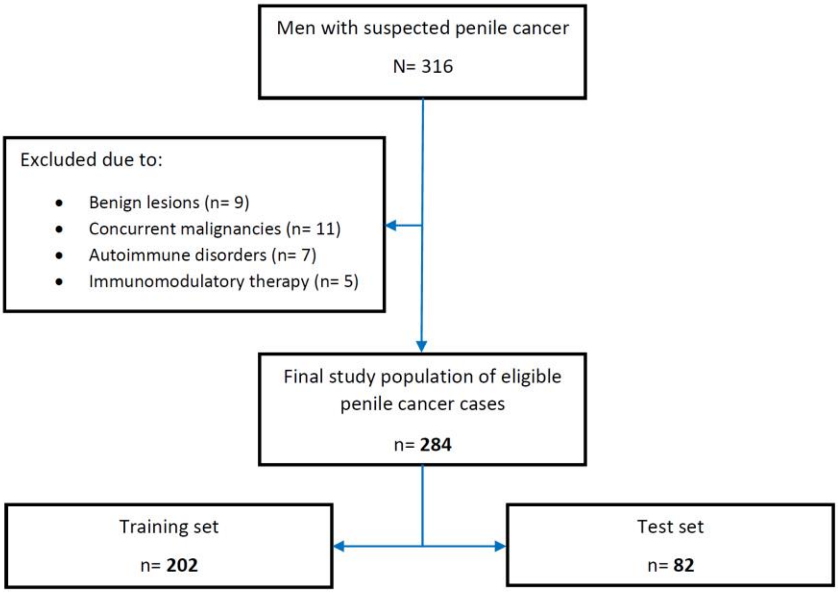

Figure 7. Flowchart outlining the study design with the inclusion and exclusion criteria.

Data collection and laboratory procedures

Clinicopathological data, including age, date of diagnosis, tumour characteristics (TNM), and HPV status, were extracted from medical records. Preoperative blood samples were collected from all patients and processed to obtain plasma, which was subsequently analysed using a bead-based multiplex immunoassay platform (Luminex Technology, Austin, TX, USA). This methodology employs color-coded microspheres conjugated with capture antibodies specific to each target protein, enabling simultaneous detection and quantification of multiple analytes within a single sample. Following incubation with plasma, target proteins bind to their respective antibody-coated beads. The Luminex instrument uses laser flow cytometry to identify each bead and quantify the associated fluorescence signal, thereby allowing reproducible and sensitive measurement of multiple soluble proteins in parallel (Figure 8). Using this approach, plasma levels of ten inhibitory immune checkpoint proteins, i.e., B- and T-lymphocyte attenuator (BTLA), indoleamine 2,3 dioxygenase (IDO), lymphocyte activation gene 3 (LAG-3), herpes virus entry mediator (HVEM), programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1) and PD-L2, T-cell immunoglobulin and mucin domain 3 (TIM-3), CD80, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4/CD152), as well as four stimulatory proteins, i.e., glucocorticoid-induced tumour necrosis factor receptor-related protein (GITR), CD27, CD28, and CD137, were simultaneously quantified.

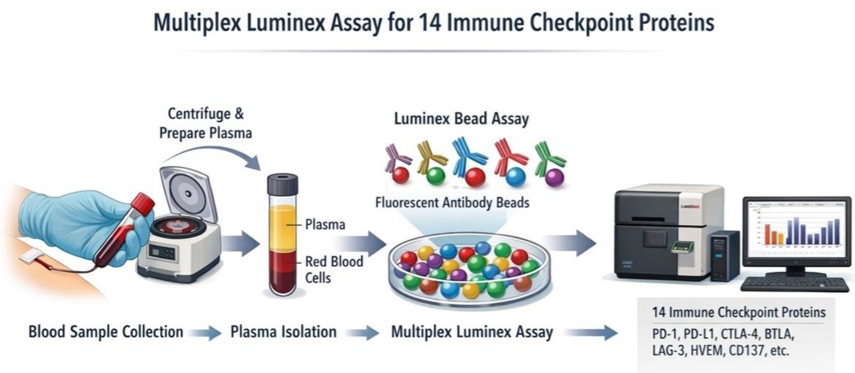

Figure 8. Workflow of multiplex Luminex assay for quantification of immune checkpoint proteins. Peripheral blood is centrifuged to separate plasma from red blood cells. The isolated plasma is then incubated with a panel of fluorescently labelled antibody-coated beads, each specific for a distinct immune checkpoint protein. Following binding, the bead–analyte complexes are analysed using a Luminex instrument, which detects bead identity and fluorescence intensity to enable simultaneous quantification of multiple targets. AI-generated image.

Statistical analysis

Only proteins detectable in >50% of samples were included in further analyses, leading to the exclusion of PD-L1. Data were Box–Cox transformed to approximate normality. Differences in the plasma concentrations of the soluble immune checkpoint proteins between the penile cancer cohort and the cancer-free controls, as well as between patients with and without lymph node metastases, were assessed using Welch's *t*-tests or ANOVA for continuous variables. Categorical variables were analysed using Chi-square or Fisher's exact tests. Multiple testing was adjusted for using the Benjamini–Hochberg false discovery rate procedure. To evaluate their predictive potential for lymph node metastases, a logistic regression model was developed in the training set and subsequently validated in the test set. Model performance was evaluated using accuracy, balanced accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under the curve (AUC) with corresponding 95% CIs.

Paper IV: Retrospective clinical cohort study

Study design and population

Paper IV is a retrospective clinical cohort study evaluating the diagnostic accuracy and morbidity of DSNB. The final study population comprised 229 penile cancer patients presenting with at least one clinically node-negative (cN0) groin, who underwent a total of 461 DSNB procedures at Örebro University Hospital, one of Sweden's two national tertiary referral centres for penile cancer (Figure 9).

Patients were recruited from Örebro University Hospital and Stockholm South General Hospital (in charge of penile cancer care in the Stockholm–Gotland region) between January 2015 and September 2024. The start of the inclusion period explicitly aligns with the national centralisation of curative penile cancer surgery in Sweden. For all included patients, the indication for DSNB was established at the national MDT conference and subsequently confirmed by a specialised urologist during the preoperative consultation.

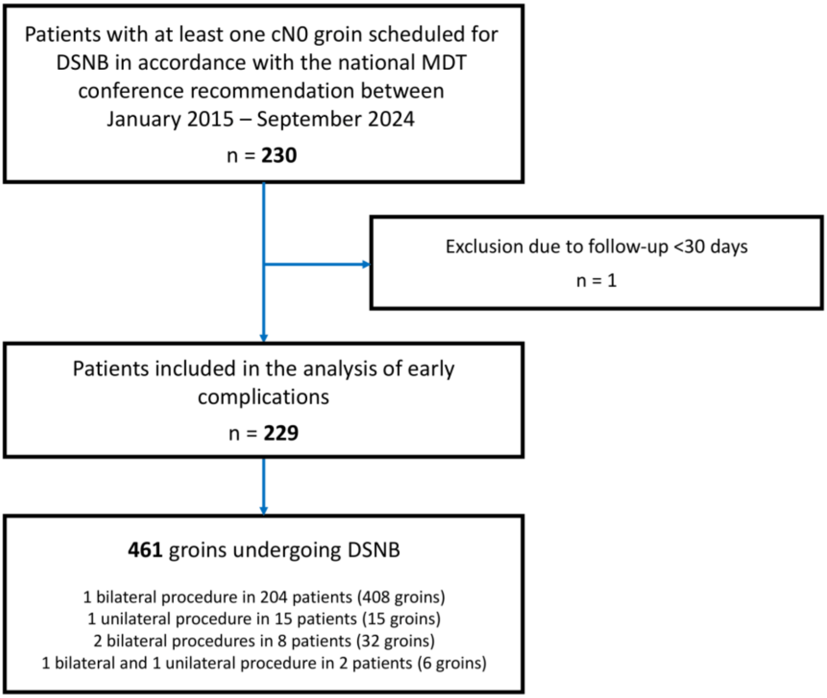

Figure 9. Flowchart depicting the study cohort, number of dynamic sentinel node biopsy (DSNB) operated groins and exclusion criteria.

Data collection and DSNB algorithm

Comprehensive clinical, surgical, pathological, and follow-up data were retrieved through structured reviews of medical records by an experienced urologist. The standardised DSNB algorithm began with a preoperative groin US, performed approximately one week prior to surgery, with the option of FNAC for any suspicious inguinal lymph nodes. Subsequently, lymphoscintigraphy utilising a 99mTc-nanocolloid tracer was performed according to either a one- or two-day protocol. This was followed by SPECT/CT imaging to ensure precise anatomical localisation of the sentinel nodes. Intraoperatively, patent blue dye was injected intradermally at the penile base to facilitate optical identification of the lymphatic drainage. The sentinel nodes were then identified and excised using a combination of a handheld gamma probe, visual inspection, and palpation.

Postoperative complications and follow-up

All postoperative adverse events were recorded per groin and graded according to the modified Clavien–Dindo (CD) classification system, further categorised into early (≤30 days) or late (>30 days) complications (100, 101). In cases where an ILND was performed in the contralateral groin, systemic complications were assigned to the DSNB procedure only if a concurrent local DSNB-related complication was present. Furthermore, groins that underwent a completion ILND following a positive DSNB were included in the early complication analysis but excluded from the late complication analysis.

For analysis, complications were grouped as mild (CD I), moderate (CD II), or severe (CD III and IV). Severe complications were defined as those requiring radiological intervention under local anaesthesia (CD IIIa), surgical intervention under general anaesthesia (CD IIIb), or admission to the intensive care unit (CD IV).

Follow-up was conducted in accordance with the recommendations of the Swedish national guidelines (56). The initial postoperative follow-up visit was generally conducted at our tertiary institution, whereas subsequent follow-up was managed at either Örebro University Hospital (18%) or Stockholm South General Hospital (82%).

Statistical analysis

The false-negative (FN) rate was calculated per groin using the standard formula: (FN / (true positive + FN)) × 100. Any regional nodal recurrence on the side of a negative DSNB procedure, in the absence of a concurrent local primary tumour recurrence, was classified as an FN event. Continuous variables were presented as means with standard deviations (SDs) or as medians with interquartile ranges (IQRs). Categorical data were analysed using chi-square tests while differences in continuous variables (e.g., BMI) were assessed utilising Welch's *t*-test. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

Results

Paper I: Risk of second HPV-associated cancers

The final study cohort comprised 1634 patients with penile cancer and 9804 penile cancer-free controls. During the follow-up period, a total of six men in the penile cancer cohort were subsequently diagnosed with a second HPV-associated malignancy: four men (0.26%) with oral cavity cancer, one (0.06%) with oropharyngeal cancer, and one (0.06%) with anal cancer. In the control group, the corresponding numbers were ten (0.10%), two (0.02%), and three (0.03%), respectively.

When comparing the risk between the two groups, patients with penile cancer demonstrated an approximately two- to threefold increased risk of developing a subsequent HPV-associated cancer. Specifically, the HRs were 2.84 (95% CI 0.89–9.06) for oral cavity cancer, 3.66 (95% CI 0.33–40.39) for oropharyngeal cancer, and 2.34 (95% CI 0.24–22.47) for anal cancer (Figure 10).

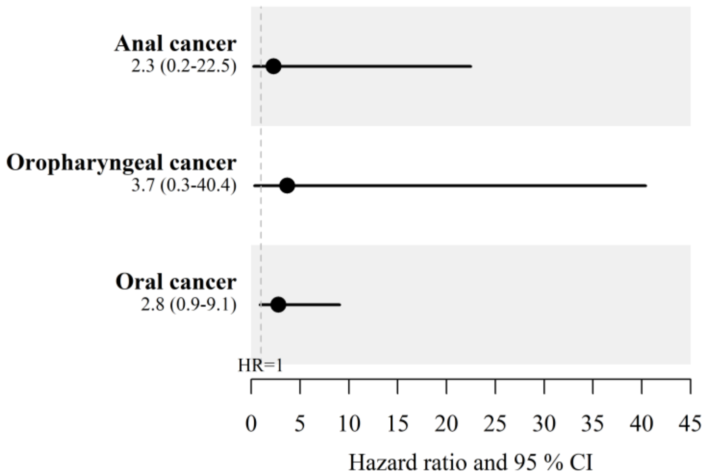

Figure 10. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for selected HPV-associated cancers in patients with a history of penile cancer compared with matched penile cancer-free controls.

A separate analysis comparing the incidence of these specific malignancies in penile cancer patients with the Swedish male general population yielded a similar pattern of increased risk. The SIRs were 2.38 (95% CI 0.64–6.09) for oral cavity cancer, 1.62 (95% CI 0.02–8.99) for oropharyngeal cancer, and 1.65 (95% CI 0.02–9.16) for anal cancer (Figure 11).

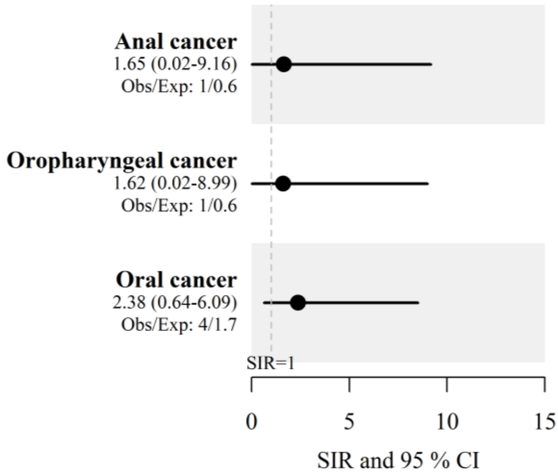

Figure 11. Standardised incidence ratios (SIRs) and 95% confidence intervals (95% CIs) for selected HPV-associated cancers in patients with a history of penile cancer compared with the Swedish male general population.

Although the absolute number of events was small, reflecting the rarity of both penile cancer and these specific subsequent malignancies, both analytical approaches consistently indicated an elevated risk of second HPV-associated cancers in this patient population.

Paper II: Long-term infectious and thromboembolic complications following lymph node dissection

The final study population comprised 364 patients with penile cancer who collectively underwent 657 ILNDs, matched with 2152 penile cancer-free controls.

Compared with the matched control cohort, patients who underwent ILND demonstrated a significantly increased risk of experiencing postoperative infectious events. This elevated risk persisted for more than 5 years postoperatively. The HRs at 12, 36, and 66 months postoperatively were 8.60 (95% CI 5.16–14.34), 4.02 (95% CI 2.65–6.09), and 1.93 (95% CI 1.11–3.38), respectively (Figure 12). In multivariable analyses, the presence of palpable nodal disease (cN+) was the only independent predictor of these subsequent infectious events, with the risk increasing proportionally with a higher cN stage.

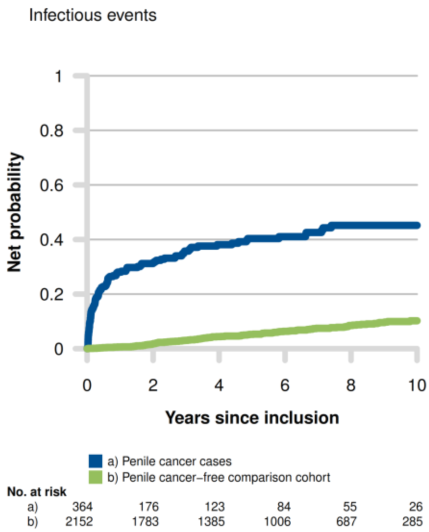

Figure 12. Net probability of infectious events in patients with penile cancer and matched penile cancer-free controls, 2000–2012.

Similarly, an elevated risk of thromboembolic events was observed and persisted for 36 months postoperatively. The HR for experiencing a thromboembolic event was the highest during the first year, at 13.51 (95% CI 6.53–27.93) at 12 months, and remained elevated at 2.12 (95% CI 1.07–4.20) at 36 months (Figure 13). Notably, this prolonged risk corresponded closely to the observed increased prescription rate of anticoagulants within the penile cancer cohort during the first postoperative years. Regarding predictors of thromboembolic events, a significant association was observed specifically with bulky nodal disease (cN3 stage). Interestingly, the addition of a PLND, performed in 108 (29.7%) of the patients undergoing ILND, did not confer any further risk of either infectious or thromboembolic events.

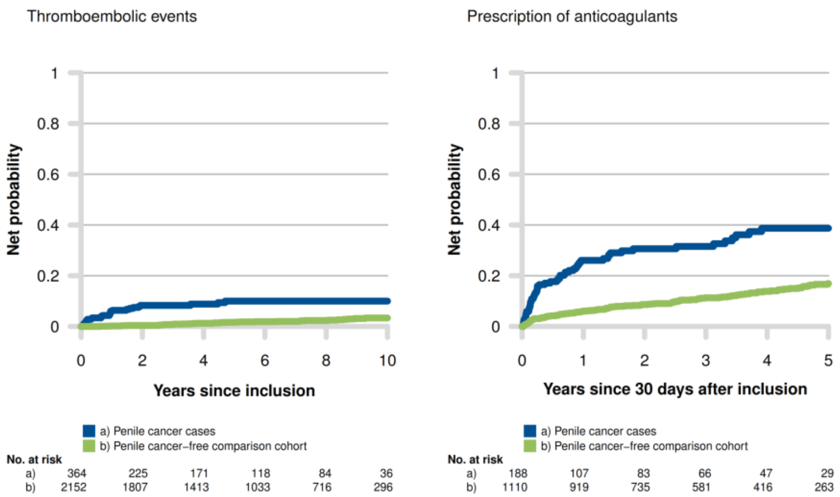

Figure 13. Net probability of thromboembolic events and prescription of anticoagulants in patients with penile cancer and matched penile cancer-free controls, 2000–2012.

Paper III: Soluble immune checkpoint proteins as predictive biomarkers for lymph node metastases

Among the 284 penile cancer patients included in the final study cohort, pathologically confirmed lymph node metastases (pN+) were present in 24.1% of the training set (*n* = 45) and 35.9% of the test set

(n = 28). No statistically significant clinicopathological differences, including age, BMI, TNM stage, and HPV status, were observed between these two sets.

Initially, the plasma levels of 14 soluble immune checkpoint proteins were measured in all patients and controls. However, PD-L1 was excluded from further analyses as it fell below the predefined detection threshold (>50% of samples), being undetectable in 96% of the samples. Consequently, a logistic regression model was developed in the training set to predict the presence of lymph node metastases based on the plasma concentrations of the 13 detectable soluble immune checkpoint proteins. In the training set, the model achieved an overall accuracy of 77.5% (95% CI 70.9–83.3) and a balanced accuracy of 54.1%. While the model demonstrated a high specificity of 99.3%, its sensitivity was notably low at 8.9%. Upon validation in the test set, the model's performance declined, yielding an accuracy of 62.2% (95% CI 50.8–72.7), a sensitivity of 17.9%, and a specificity of 85.2%. The area under the curve (AUC) was 67.1% (95% CI 58.0–76.3) in the training set and 54.2% (95% CI 40.3–68.0) in the test set, indicating limited generalisability and poor overall predictive capacity for identifying lymph node metastases (Table 3, Figure 14).

Table 3. Results of the prediction model for lymph node metastases based on 13 soluble immune checkpoint proteins in penile cancer patients divided into training and test sets.

Dataset	Accuracy % (95% CI)	Balanced ACC %	PPV % (95% CI)	NPV % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Training set (n=202)	77.5 (70.9 – 83.3)	54.1	80.0 (31.4 – 97.2)	77.5 (75.9 – 79.1)	8.9 (2.5 – 21.2)	99.3 (96.1 – 99.9)
Test set (n=82)	62.2 (50.8 – 72.7)	51.5	38.5 (18.3 – 63.3)	66.7 (62.1 – 71.2)	17.9 (6.1 – 36.9)	85.2 (72.9 – 93.4)

ACC = Accuracy, CI = Confidence interval, NPV = Negative predictive value, PPV = Positive predictive value

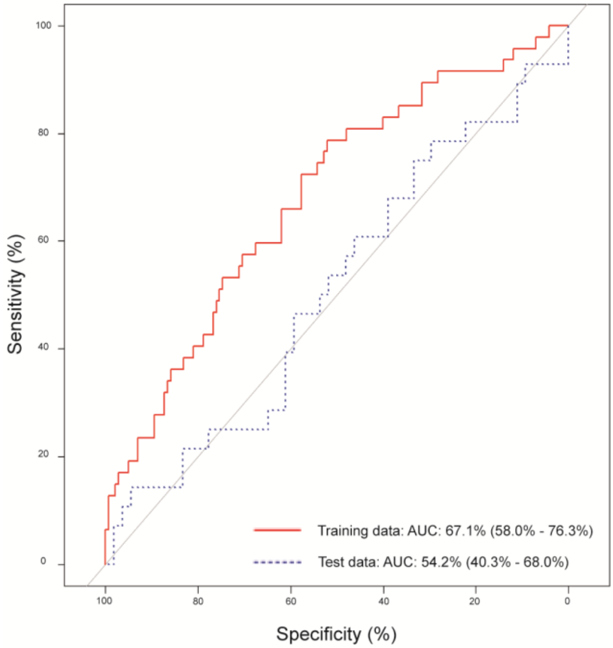

Figure 14. Area under the curve (AUC) for the proposed lymph node metastases prediction model, built using the training data and validated on the test data.

To further explore the model's limited predictive performance, the plasma levels of the 13 detectable soluble immune checkpoint proteins were compared between patients with and without lymph node metastases within the training set. No statistically significant differences were observed for any of the analysed proteins, nor did stratification by pT stage, tumour grade, or HPV status reveal any significant differences.

However, in a secondary sub-analysis comparing plasma levels between the penile cancer cohort and the 45 cancer-free controls, four inhibitory soluble immune checkpoint proteins, specifically IDO, TIM-3, CD80, and CTLA-4, were detected at significantly higher mean concentrations in patients with penile cancer.

Paper IV: Diagnostic accuracy and morbidity of dynamic sentinel node biopsy

During follow-up of the 229 penile cancer patients included in the final study cohort, primary tumour recurrence occurred in 21 patients. Ten of these patients underwent repeat DSNB, performed either bilaterally ($n = 8$) or unilaterally ($n = 2$) due to a prior unilateral ILND. Consequently, the 229 patients underwent a total of 239 DSNB surgeries, which collectively comprised 461 operated groins (DSNB procedures). Of these 239 surgeries, 20 (8.3%) were performed after the primary tumour surgery (delayed DSNB). The overall median length of stay was one postoperative day (IQR 1–1). Delayed DSNB was mostly performed as day surgery, with a median length of stay of zero days (IQR 0–1). Baseline demographic, clinical, and surgical characteristics of the cohort are presented in Table 4.

A total of 1064 lymph nodes were excised, corresponding to a median of two sentinel nodes (IQR 1–3) per groin. Metastases were detected in 60 lymph nodes (5.6%), involving 51 groins (11.1%) across 44 patients (19.2%). Notably, micrometastases (≤2 mm) accounted for 55% of the positive lymph nodes.

Over a median follow-up of 34 months (IQR 13–57), the overall FN rate per groin was 14.5%, accounting for cN0 groins correctly upstaged by preoperative US with positive FNAC (i.e., metastases detected prior to the surgical step of DSNB but within the DSNB staging algorithm). A unilateral FN DSNB was observed in 10 patients. Two of these FN events occurred 5 and 9 months, respectively, after a repeat DSNB performed for a primary tumour recurrence. In the remaining eight patients, who had undergone a single DSNB surgery, nodal recurrences were detected at 5, 7, 10, 12, 13, 19, 25, and 43 months postoperatively, and were therefore classified as FN procedures. The 2-year probability of a negative DSNB procedure becoming false-negative was estimated at 2%.

Table 4. Patient, tumour, and surgical characteristics.

Patient characteristics	
Patients, n	229
Surgeries, n	239
Age, years, median (IQR)	71 (63 – 77)
BMI, kg/m^2, mean (SD)[1]	27.3 (4.5)
Obese BMI ≥30, n (%)	53 (23.1)
Diabetes, n (%)	53 (23.0)
Smoking status, n (%)	
Never	105 (45.9)
Former	91 (39.7)
Current	33 (14.4)
ASA, n (%)	
1	124 (54.1)
2	71 (31.0)
3	28 (12.2)
4	6 (2.6)
pT-stage, n (%)	
PeIN	13 (5.7)
T1	101 (44.1)
T2	73 (31.9)
T3	42 (18.3)
Grade, n (%)	
G1	27 (11.8)
G2	116 (50.7)
G3	73 (31.9)
HPV-positive, n (%)[2]	116 (50.7)
Follow-up, months, median (IQR)	34 (13 – 57)
Surgical characteristics and outcomes	
DSNB procedures, n	461
Primary tumour surgery, n (%)	
Organ-sparing	151 (65.9)
Amputation	78 (34.1)
Number of removed LNs, n	1064
Number of removed LNs per groin, median (IQR)	2 (1 – 3)
Number of pathologic LNs, n (%)	60 (5.6)
Delayed DSNB, n (%)	20 (8.4)
2-day protocol, n (%)	136 (56.9)
Length of stay, postoperative days, median (IQR)	1 (1 – 1)
Length of stay - delayed DSNB, postoperative days, median (IQR)	0 (0 – 1)
Readmission, n (%)	33 (13.8)

[1] Missing information n = 1, [2] Missing information n = 6

IQR = Interquartile Range, BMI = Body Mass Index, SD = Standard Deviation, ASA = American Society of Anaesthesiologists (ASA) Physical Status Classification System, pT = Pathological Tumour stage, HPV = Human papillomavirus, DSNB = Dynamic Sentinel Node Biopsy, LN = Lymph Node

DSNB-related complications occurred in 14.8% of the operated groins (68/461). The vast majority of these were early complications (≤30 days), which constituted 84% of all adverse events and were predominantly classified as mild to moderate (CD grades I–II). Severe early complications were predominantly classified as CD grade IIIa, primarily requiring ultrasound-guided drain placements under local anaesthesia for lymphoceles, combined with antibiotic therapy in cases of infection (Table 5). Late complications (>30 days) were rare, occurring in only 2.6% of the groins (11/418), with infectious events being the most common manifestation (72.7%) (Table 6). Overall, readmissions due to postoperative complications occurred in 33 patients (13.8%), most commonly for intravenous (i.v.) antibiotic therapy.

When evaluating risk factors for postoperative morbidity, the lymph node yield was the only variable significantly associated with an increased complication rate. Complications were observed in 24.1% of groins where three or more lymph nodes were excised ($n = 162$), compared with 9.4% in groins where one or two lymph nodes were excised ($n = 299$) ($p < 0.001$). Age, ASA physical status, BMI, smoking, and diabetes showed no significant association with DSNB-related morbidity.

Table 5. Summary of 57 early postoperative complications in 461 groins.

Complication	Groins, n (%)	Treatment	Treatment, n (%)	Clavien-Dindo
Wound infection	15 (3.3)			
		Antibiotics (oral)	8 (53.3)	II
		Antibiotics (i.v.)	7 (46.7)	II
Lymphocele	12 (2.6)			
		Drainage	8 (66.7)	I
		US drain	4 (33.3)	IIIa
Infected lymphocele	29 (6.3)			
		Drainage + Antibiotics (oral)	2 (6.9)	II
		Drainage + Antibiotics (i.v.)	5 (17.2)	II
		US Drain + Antibiotics (oral)	2 (6.9)	IIIa
		US Drain + Antibiotics (i.v.)	19 (65.5)	IIIa
		Surgical intervention + Antibiotics (i.v.)	1 (3.4)	IIIb
Sepsis	1 (0.2)			
		Antibiotics (i.v.)	1 (100.0)	IVa

i.v. = intravenous, US = Ultrasound

Table 6. Summary of 11 late postoperative complications in 418 eligible groins.

Complication	Groins, n (%)	Treatment	Treatment, n (%)	Clavien-Dindo
Lymphocele	1 (0.2)			
		US drain	1 (100.0)	IIIa
Infected lymphocele	4 (1.0)			
		Drainage + Antibiotics (i.v.)	2 (50.0)	II
		US Drain + Antibiotics (i.v.)	2 (50.0)	IIIa
Erysipelas	4 (1.0)			
		Antibiotics (oral)	4 (100.0)	II
Lower extremity lymphoedema	2 (0.5)			
		Conservative	2 (100.0)	I

i.v. = intravenous, US = Ultrasound

Discussion

The overall aim of this thesis was to optimise the clinical management of patients with penile cancer by mapping long-term oncological and surgical risks on a population-based level, evaluating the diagnostic accuracy and morbidity of DSNB, and exploring the potential of novel, non-invasive predictive biomarkers obtained from liquid biopsies. Because penile cancer is a rare disease in which patient survival decreases dramatically once metastatic spread has occurred, historical treatment strategies have often prioritised survival at any cost. Consequently, these approaches have frequently led to aggressive interventions, resulting in significant overtreatment, severe physical and psychological stigmatisation, and lifelong morbidity. By combining robust national registry data with translational research on prospectively collected samples and rigorously evaluated clinical cohorts, the findings of this thesis highlight the ultimate clinical challenge of achieving an optimal balance between maximising oncological control and preserving the patient's long-term quality of life.

The HPV burden and the risk of second malignancies

A fundamental aspect of penile cancer survivorship is understanding the long-term risks associated with the disease's viral aetiology. Despite the rarity of penile cancer, it shares a common aetiology with several much more prevalent malignancies through HPV, the most common sexually transmitted infection globally (102). Approximately 50% of all penile cancers are directly related to oncogenic HPV types (primarily HPV16 and 18) (22, 24). In **Paper I**, we found indications that men diagnosed with penile cancer have an approximately two- to threefold increased risk of developing a second HPV-associated cancer in the oral cavity, oropharynx, or anal canal later in life. Although these findings are based on small numbers yielding wide confidence intervals, our risk assessment utilised two distinct analytical approaches that produced consistent estimates.

These results align well with the results of previous large population-based studies. For instance, Suk et al. demonstrated that the overall risk of developing any HPV-associated cancer is significantly higher following a primary HPV-associated malignancy compared with a non-HPV-associated one. Specifically for penile cancer patients, they reported a twofold increased risk of subsequent oropharyngeal cancer, which corresponds closely with the estimates in **Paper I** (**31**). Studies by Nelson and Lai and by Neumann et al. have also shown an elevated risk of HPV-associated malignancies in patients who previously had an HPV-associated anogenital cancer (103, 104). In addition, pooled results of a systematic review and meta-analysis by Gilbert et al. demonstrated that penile cancer patients are at an increased risk of oropharyngeal cancer, yielding estimates broadly in line with our results (105).

The underlying mechanism of this increased risk is likely multifactorial. Firstly, the inability of the individual's immune system to clear the acquired oncogenic virus can lead to persistent infections across multiple susceptible anatomical sites. Secondly, behavioural risk factors, such as the number of sexual partners and specific sexual practices, may contribute to the virus exposure in different anatomical regions (106). Whether driven by biological susceptibility or behavioural risk, these patients carry a lifelong elevated risk.

Although the absolute number of second cancers was relatively low in our cohort (only six patients in the penile cancer group developed a second HPV-associated cancer), the elevated relative risk has important clinical implications for follow-up. Currently, the surveillance of penile cancer patients focuses predominantly on local and regional recurrences. Our findings underscore the potential need for a broader clinical approach to enable the early detection of other premalignant lesions, alongside targeted preventive measures such as smoking cessation. Furthermore, these findings provide strong scientific support for gender-neutral HPV vaccination programs. This highly effective, safe, and relatively simple preventive measure has the potential to drastically reduce the incidence of both primary penile cancer and the subsequent burden of second HPV-associated malignancies (12, 13).

Long-term morbidity following lymph node dissection

Lymph node status remains the single most important prognostic factor in penile cancer (5). Historically, radical ILND has been the standard of care for both diagnosis and therapy, as early and radical excision of lymph node metastases drastically improves cancer-specific survival compared with delayed intervention. In fact, 3-year cancer-specific survival declines from 84% to a mere 35% if ILND is deferred until nodal disease becomes clinically palpable during follow-up (49). However, this survival benefit must be weighed against the risk of substantial overtreatment, given that occult lymph node metastases are present in only 20–25% of clinically node-negative patients (6, 50). Moreover, this extensive procedure is associated with substantial morbidity, with complication rates reaching up to 70% (52, 107).

The majority of previous publications have primarily focused on early postoperative complications occurring within the first 30 days. When assessed, late complications were usually defined as any adverse event emerging beyond this initial 30-day period; however, knowledge regarding true long-term complications has remained limited.

Paper II demonstrated that surgical morbidity is present not only during the early postoperative period but extends several years into the future. To capture this long-term burden, we utilised a population-based approach relying on high-quality Swedish national registries with exceptional coverage, validity, and the ability to link data using the unique personal identity number. Our results showed that the overall risk of infectious and thromboembolic events one year after ILND was 8 and 13 times higher, respectively, compared with a penile cancer-free control group. Furthermore, men undergoing ILND faced a significantly elevated risk of infectious complications for more than five years postoperatively. Additionally, an elevated risk of thromboembolic events persisted for up to three years, corresponding closely to the observed increased prescription rate of anticoagulants in this cohort during the first postoperative years.

These findings align with and complement other large series evaluating the morbidity of lymph node dissection. Gopman et al. observed that 55.4% of patients undergoing ILND experienced at least one postoperative complication, with wound infections being the most common (52). Similarly, Stuiver et al. reported an overall complication rate of 58% following ILND, with wound infections occurring in 43% of patients (51). Recently, Jeanne-Julien et al. identified early complications in 69% of patients operated with radical ILND, noting lymphocele formation as the most common early complication and leg lymphoedema as the most common late complication (107).

While a substantial proportion of reported early complications are wound related, mainly due to the nature of the open dissection, lymphoedema remains the most prevalent late-onset adverse event. This is because the extirpation of the lymph nodes results in a chronic disruption of the lymphatic drainage from the lower extremities and genitalia. This diminished lymphatic drainage can, in turn, predispose patients to recurrent erysipelas and cellulitis.

Regarding thromboembolic complications, it is well established that patients with underlying malignancies are generally at greater risk, with the highest risk occurring during the first year following the cancer diagnosis (108). In accordance with our findings, Blom et al. found that the relative risk seems to decrease considerably two years after diagnosis but remains elevated compared with cancer-free individuals (109). Furthermore, surgery itself represents an independent risk factor for this severe complication. A series of previous studies have reported rates of thromboembolic events between 4% and 14% after ILND for penile cancer (82, 83, 110).

The nodal stage of the disease is also a critical factor, as a higher nodal stage inherently increases the complexity, extent, and duration of surgery. Our results indicate that the presence of palpable metastases (cN+) predicts an increased risk of infectious events, while bulky nodal disease (cN3) is a predictor of thromboembolism.

To mitigate these surgical risks, minimally invasive techniques such as video-endoscopic inguinal lymphadenectomy (VEIL) and robot-assisted VEIL (RA-VEIL) are increasingly being explored. While recent meta-analyses demonstrate that these approaches yield similar oncological outcomes and significantly reduce early wound complications compared with open ILND, they fail to decrease lymphatic-related complications (e.g., lymphoceles and lymphoedema) (90, 111). This highlights that lymphatic morbidity is inherently tied to the number of lymph nodes harvested and the unavoidable disruption of the lymphatic vessels, regardless of the surgical access route.

Ultimately, early radical lymph node dissection remains the gold standard for the treatment of patients with confirmed regional metastases. However, these compelling long-term morbidity data provide a powerful argument against using ILND for purely diagnostic purposes in cN0 patients, underscoring the absolute necessity of less invasive surgical staging methods.

Strengths and limitations of register-based studies

The central strength of the epidemiological findings in this thesis lies in the population-based design facilitated by the Swedish national healthcare registries. The high coverage, longitudinal data, and ability to link records seamlessly for detailed analyses minimise selection bias and provide a representative reflection of clinical reality, yielding results that are highly generalisable to everyday practice. However, register-based research inherently relies on the variables available within the databases, which introduces certain limitations.

A primary limitation in **Paper I** was the lack of information on confirmed HPV status, precluding the assessment of subsequent cancer risks stratified by HPV status. This may have resulted in the dilution of the observed associations. Furthermore, information on other established risk factors for HPV-associated cancers, such as smoking history and specific sexual practices, was unavailable in the registries, limiting our ability to control for these confounders.

Similarly, **Paper II** carries limitations related to a lack of detailed clinical data. First, even though modified ILND is associated with less postoperative morbidity compared with radical ILND, both procedures are classified under the same intervention code in the Swedish registers. However, modified ILND is strictly a diagnostic procedure and should be complemented with radical ILND if metastases are found. Moreover, with the introduction of DSNB, the number of ILND procedures performed in the purely diagnostic setting has declined dramatically. Second, while we were able to adjust for multiple factors potentially associated with an increased risk of infections and thromboembolism, no information was available on smoking, BMI, and specific oncological treatments in the utilised dataset.

Dynamic sentinel node biopsy: Balancing diagnostic accuracy and surgical morbidity

Given the high morbidity associated with diagnostic ILND, the limited sensitivity of current imaging modalities for detecting occult lymph node metastases, and the lack of validated predictive biomarkers, invasive lymph node staging remains indispensable (6, 52, 112). DSNB, a minimally invasive staging procedure, has become the recommended approach for cN0 intermediate- and high-risk penile cancer (≥T1G2) (6, 56). In **Paper IV**, we evaluated the performance of DSNB within the framework of a national tertiary referral centre. The results demonstrated that DSNB effectively detects nodal dissemination, identifying metastases in 11.1% of the evaluated groins, corresponding to 19.2% of patients. This ultimately spares more than 80% of cN0 patients from the untoward consequences of an unnecessary ILND. Moreover, more than half of the positive lymph nodes were micrometastases, underscoring the clinical relevance of DSNB as a staging procedure.

A central finding in **Paper IV** was the highly favourable morbidity profile of DSNB compared with diagnostic ILND. During the entire follow-up period, DSNB-related complications occurred in 14.8% of groins and were predominantly mild to moderate (CD I–II), such as lymphoceles, wound infections, or infected lymphoceles manageable with conservative or minor interventions. The vast majority of these events occurred within 30 days of surgery. However, interpreting complication severity using standard grading systems warrants caution in this setting. Although some events were classified as CD IIIa, these primarily involved US-guided lymphocele drainage under local anaesthesia. While meeting the formal criteria for a severe complication, the actual clinical burden was limited, with several cases managed entirely in an outpatient setting. Consequently, categorically grouping all CD III–IV events as severe overestimates the true morbidity of DSNB, as genuine high-severity complications (CD IIIb–IV) were exceedingly rare in our cohort.

Nevertheless, we identified a critical association between the surgical extent and morbidity: when three or more lymph nodes were excised (35% of groins), the complication rate surged significantly from 9.4%

to 24.1%. These findings are consistent with the findings of Wever et al., who identified the number of removed lymph nodes as a main predictor of postoperative complications, reporting complication rates in line with our results (58). A similar trend was also previously reported by Dimopoulos et al (113). Our data therefore provide a compelling argument for targeted dissection restricted solely to true sentinel nodes. Removing additional lymph nodes unnecessarily diminishes the primary advantage of the method and exposes the patient to increased surgical risks.

Despite its advantages, DSNB is not without limitations. In our cohort, we observed an FN rate of 14.5% over a median follow-up of 34 months. To interpret the FN rate accurately, it must be contextualised within our stringent DSNB algorithm. In our centralised pathway, all patients routinely undergo preoperative groin US, with the option of FNAC for any suspicious lymph nodes. Contemporary data indicate that this systematic approach can effectively filter out approximately 10–13% of patients with detectable metastases, directing them straight to therapeutic ILND and reducing the number of true-positive DSNB procedures (63, 64). Furthermore, our extended follow-up successfully captured very late nodal recurrences, occurring up to 43 months postoperatively. Inherently, studies with longer follow-up periods detect more late nodal recurrences, decreasing the apparent DSNB accuracy over time, despite comparable early performance. Hence, direct comparisons of FN rates between series require careful interpretation.

A recent systematic review and meta-analysis evaluating the diagnostic accuracy of DSNB reported a pooled sensitivity of 87% across 28 studies, highlighting both the overall value of DSNB and the heterogeneity in reported diagnostic performance (114). For instance, a high-quality contemporary study by Wever et al. evaluated DSNB performed in 644 penile cancer patients with a median follow-up of 23 months, reporting an FN rate of 8.3% (58). Given that two of our FN events occurred more than 24 months postoperatively, differences in follow-up duration are likely to contribute to inter-study variability. Methodological differences play a similar role. In the DaPeCa-1 trial, Jakobsen et al. assessed 222 penile cancer patients undergoing DSNB; however, groin US with FNAC on indication was not applied systematically to all cN0 patients, but rather selectively. As a result, a proportion of metastases that could have been detected prior to surgery were instead identified during DSNB and counted as true positives, artificially lowering the apparent FN rate to 10.8% (59). In a different series approach, Lam et al. performed US groin assessment in all 264 penile cancer patients, but FNAC-positive patients still proceeded to DSNB and were subsequently included in the FN calculations, resulting in an exceptionally low FN rate of 5% (60).

Our findings underscore that a certain FN risk is an unavoidable compromise when attempting to escape the severe morbidity of diagnostic ILND. For DSNB to function safely, high surgical volume, multidisciplinary expertise, and an extremely vigilant, long-term clinical surveillance program are required in order to effectively balance oncological control with the preservation of the patient's quality of life.

The fundamental strengths of this study include its large cohort, standardised DSNB protocol within a centralised, multidisciplinary-driven pathway, and comprehensive medical record review that incorporated follow-up data from the referring hospitals. Furthermore, the extended follow-up enabled the detection of late nodal recurrences, alongside detailed complication reporting utilising CD grading with early and late stratification. However, certain limitations must be acknowledged, primarily its retrospective design and the potential for heterogeneity in postoperative surveillance outside the tertiary centre. Selection bias is also a possibility, as follow-up data were primarily available from two hospitals (Örebro University Hospital and Stockholm South General Hospital). This could potentially skew the estimates of DSNB positivity and the FN rate if true-positive cases are underrepresented, whereas FN cases are likely captured due to the mandatory national MDT reviews and subsequent referrals for salvage ILND to a tertiary centre.

Beyond surgical staging: Soluble immune checkpoint proteins as predictive biomarkers for lymph node metastases

Although DSNB has substantially refined the staging of cN0 penile cancer, it remains an invasive surgical procedure. The ultimate clinical goal is to develop highly sensitive, non-invasive tools to more accurately stratify patients. This would reduce unnecessary surgical interventions for patients at low risk of metastatic spread, while ensuring that high-risk patients with aggressive tumours receive definitive treatment without unwarranted delay. In **Paper III**, we explored the potential of liquid biopsies in penile cancer by evaluating a broader panel of soluble immune checkpoint proteins as predictive biomarkers of lymph node metastases. Soluble immune checkpoint proteins have emerged as promising non-invasive biomarkers in various malignancies, but their prognostic role in penile cancer has not previously been investigated (44).

To investigate their predictive capacity for lymph node metastases, we analysed plasma samples from 284 penile cancer patients. The cohort was divided into a training set (n = 202) for model development and an independent test set (n = 82) for validation. Our logistic regression model, which incorporated 13 detectable soluble immune checkpoint proteins, demonstrated limited clinical utility in predicting lymph node metastases. Although the model achieved high specificity in the training set (99.3%), its sensitivity was very low (8.9%), resulting in only modest overall accuracy (77.5%). The model's performance declined further when validated in the independent test set, highlighting its limited predictive reliability. Incorporating established clinicopathological variables, such as pT stage and tumour grade, provided only a modest improvement in sensitivity, and the overall predictive capacity remained insufficient for clinical application. Furthermore, no significant differences in the plasma levels of soluble immune checkpoint proteins were observed between patients with and without confirmed lymph node metastases. These results indicate that circulating systemic levels of soluble immune checkpoint proteins alone may not adequately reflect the local immune dynamics that drive metastatic progression to regional lymph nodes.

Our findings contrast with those of previous studies evaluating soluble immune checkpoint proteins in other solid malignancies. For example, research in renal cell carcinoma, pancreatic adenocarcinoma, and osteosarcoma has demonstrated significant correlations between elevated soluble immune checkpoint proteins, such as TIM-3, BTLA, and PD-L1, and advanced disease, tumour progression, or reduced survival (115-117). These discrepancies may be attributed to methodological differences, including variations in assays and detection techniques. Alternatively, they suggest that circulating immune checkpoint protein profiles reflect tumour aggressiveness in a cancer-specific manner, indicating that biomarker signatures cannot be universally applied across different tumour types.

Despite the limited value in predicting regional metastases, **Paper III** provided important pathophysiological insights into tumour-induced immunomodulation. Compared with penile cancer-free controls, patients with penile cancer exhibited significantly higher plasma concentrations of four inhibitory soluble immune checkpoint proteins (IDO, TIM-3, CD80, and CTLA-4). These inhibitory immune checkpoints are key regulators that limit immune activation, and their concurrent elevation suggests a distinct shift towards a systemic immunosuppressive environment. This indicates that penile tumours exploit multiple immune regulatory pathways to evade immune surveillance, and that this systemic immunomodulation likely occurs early in disease development, independent of metastatic tumour burden.

The discrepancy between our liquid biopsy-based findings and previous tissue-based studies, where biomarkers such as PD-L1 are frequently overexpressed and associated with adverse outcomes, underscores key methodological differences (40-42, 118). Circulating immune checkpoint profiles are strongly influenced by systemic inflammation and individual immune variability, which may prevent them from accurately reflecting the local immune interactions occurring within the tumour microenvironment. Notably, the absence of detectable PD-L1 in our cohort likely reflects the detection limits of the multiplex assay rather than a true biological absence.

The methodological strength of **Paper III** is reinforced by its relatively large cohort size, inclusion of a cancer-free control group, and use of both a training set and an independent test set to validate the prediction model. However, the study was primarily limited by the small absolute number of lymph node-positive cases, which restricted statistical power and contributed to class imbalance. Ultimately, while the profiling of soluble immune checkpoint proteins cannot currently replace surgical staging, combining these liquid biopsies with targeted tissue-based assessments may be crucial for clarifying their prognostic value and guiding the development of future targeted immunotherapeutic strategies in penile cancer.

Ethical considerations

All research within this thesis was conducted in accordance with the Declaration of Helsinki. The studies (**Papers I–IV**) have all been evaluated and approved by the Swedish Ethical Review Authority.

Papers I, II, and **IV** are retrospective in nature. **Papers I** and **II** are based on data from Swedish national health and quality registries. Written information regarding the Swedish quality registries, including the National Penile Cancer Register (NPECR), is provided to all patients diagnosed with penile cancer, offering them the opportunity to opt out of data registration. Because the datasets provided to the researchers for these studies were pseudonymised, the requirement for explicit informed consent from the individuals in the study cohorts was waived by the Ethical Review Authority. **Paper IV** is a retrospective study based on pre-existing data extracted from medical records. No additional clinical examinations or treatments were performed on the included patients.

Paper III is based on a prospectively enrolled patient cohort with inclusion completed in 2022. For this study, all included patients received comprehensive oral and written information about the project and provided written informed consent prior to participation.

While collecting data from medical records and national registries carries a theoretical risk of violating personal privacy and integrity, we mitigated this by strictly limiting data extraction to information directly relevant to the study objectives. We consider this risk to be minimal, as all identifiable data were handled with extreme caution and strict confidentiality. Furthermore, all analyses in **Papers I–IV** were performed on pseudonymised data, and the results are presented exclusively at a group level, precluding the identification of any individual patient. Given the high median age at diagnosis in penile cancer, a proportion of the included patients were already deceased at the time of data analysis, further motivating the register-based approach without re-consenting.

Regarding physical risks, **Paper III** required blood samples in order to analyse immune checkpoint proteins. Although blood sampling may cause mild, temporary discomfort, this was minimised by predominantly drawing the research samples in conjunction with routine clinical blood tests, thereby avoiding additional needle sticks.

We conclude that these projects posed no risk to the participants' physical health. The potential clinical and scientific benefits of obtaining concrete, generalisable results far outweigh the minor theoretical risks to privacy. These studies are essential for evaluating established clinical practices as well as potential future diagnostic and therapeutic modalities. Because the analyses were conducted retrospectively or after a follow-up period, the findings may not directly or immediately benefit the individual participants.

Nevertheless, the impact of this research extends beyond merely contributing to updated clinical guidelines. By advancing our understanding of risk stratification, novel predictive biomarkers, and long-term surgical morbidity, these studies pave the way for a more personalised approach to patient care. Ultimately, this work has the potential to spare future patients from unnecessary, high-risk surgical interventions, optimise postoperative surveillance, and significantly improve both the long-term clinical management and quality of life of men facing penile cancer.

Conclusions

The findings of this thesis contribute to a deeper understanding of the long-term consequences of penile cancer and its treatment, while also evaluating and exploring methods for improved nodal staging. The specific conclusions are as follows:

Paper I

Based on nationwide register data, our findings indicate that patients with penile cancer are at increased risk of developing a second HPV-associated cancer, particularly in the oral cavity, oropharynx, and anal canal. While the rarity of these cancers warrants larger studies with extended follow-up, an improved understanding of this risk is crucial for reducing subsequent morbidity and mortality through targeted monitoring and preventive strategies.

Paper II

Although lymph node dissection remains the gold standard for treating regional metastases, it is associated with a substantial and prolonged morbidity burden. Population-based data reveal that the risk of infectious and thromboembolic events remains significantly elevated for more than five and three years postoperatively, respectively. Improved awareness of these long-term adverse events is essential for early detection and intervention.

Paper III

A panel of 14 soluble immune checkpoint proteins demonstrated limited clinical utility for predicting lymph node metastases due to low sensitivity and modest accuracy. However, four inhibitory soluble immune checkpoint proteins (IDO, TIM-3, CD80, and CTLA-4) were found at significantly higher levels in penile cancer patients than in cancer-free controls. This suggests that systemic immunosuppression is associated with tumour presence, independent of metastatic burden. Future studies assessing larger cohorts and integrating both tissue-based and soluble immune checkpoint proteins are warranted to clarify their prognostic significance.

Paper IV

Within a centralised, multidisciplinary care pathway, DSNB proved highly effective, sparing more than 80% of clinically node-negative patients from the severe morbidity of diagnostic ILND. DSNB-related morbidity was generally low, with high-severity complications being exceptionally rare. However, a higher lymph node yield was directly associated with increased morbidity, strongly underscoring the critical importance of the targeted excision of true sentinel nodes. The observed false-negative rate of 14.5% needs to be interpreted within the context of the diagnostic algorithm and follow-up duration.

Future perspectives

Despite significant advances in the diagnostics and treatment of penile cancer, the key mechanisms driving tumour initiation, progression, and metastatic dissemination remain elusive. A major clinical challenge persists in the detection of occult lymph node metastases, as currently available imaging modalities lack sufficient sensitivity (112). Consequently, clinically node-negative patients continue to be stratified into risk groups based solely on the histopathological features of the primary tumour (6, 119).

However, the present risk-stratification model, in which surgery remains the cornerstone of both diagnostics and treatment, requires an overhaul. While DSNB offers high sensitivity and significantly reduces morbidity compared with diagnostic ILND, there is a clear need to further optimise diagnostic accuracy and minimise complication rates (114). Clinical data suggest a direct correlation between the number of excised lymph nodes and the risk of postoperative complications (58, 113). The fundamental principle of DSNB is to selectively identify and excise only the true primary draining lymph nodes from the primary tumour. Yet, precisely differentiating true sentinel nodes from secondary draining lymph nodes remains a clinical challenge, often resulting in the excision of multiple nodes. Refining the intraoperative identification of true sentinel nodes is therefore a crucial area for future optimisation.

Rapid progress in molecular biology and the genetic mapping of tumours offers hope for the discovery of more precise prognosticators. Current research is increasingly shifting focus toward minimally invasive molecular diagnostics, exploring the possibilities of liquid biopsies, and evaluating the significance of soluble immune checkpoint proteins and circulating tumour DNA (ctDNA) (120-122). The ability to utilise these biomarkers for non-invasive monitoring, early detection of occult metastases, and real-time assessment of tumour dynamics could profoundly transform the management of penile cancer.

Identifying such novel biomarkers to accurately stratify patients with aggressive disease and an increased risk of lymph node metastases is of utmost importance. It would improve survival outcomes while simultaneously avoiding the decline in quality of life associated with surgical overtreatment. Furthermore, integrating precise molecular staging into the current stepwise diagnostic approach could prevent delays in identifying patients who would benefit from more aggressive treatment and neoadjuvant oncological therapy, ensuring that the ideal therapeutic window is not missed.

In addition to advances in molecular research, a broader preventive perspective is necessary. An improved understanding of the risks of second cancers related to persistent HPV infection and cross-infection may reduce subsequent morbidity and mortality. The adoption of gender-neutral HPV vaccination policies (already implemented in several countries including Australia, the United States, Portugal, Switzerland, and the Nordic countries) represents a bold, highly effective long-term strategy to fundamentally reduce the global burden of HPV-associated diseases (123, 124).

Populärvetenskaplig sammanfattning

Peniscancer är en ovanlig cancerform som i Sverige drabbar omkring 150 män årligen. Sjukdomens prognos och patientens överlevnad är starkt kopplade till huruvida tumören har spridit sig till lymfkörtlarna i ljumskarna. Att med hög precision fastställa lymfkörtelstatus är därför helt avgörande för valet av behandling. Samtidigt utgör denna diagnostik en stor klinisk utmaning, eftersom dagens bilddiagnostik (som datortomografi eller ultraljud) saknar tillräcklig känslighet för att upptäcka mikroskopiska metastaser.

Historiskt har rutinen varit att utföra en diagnostisk lymfkörtelutrymning (ILND) i ljumskarna på patienter med förhöjd risk för spridning. Ingreppet är dock förknippat med betydande komplikationer. För att förstå omfattningen av detta utvärderade vi den långsiktiga sjukligheten efter denna typ av kirurgi med hjälp av nationella registerdata. Resultaten påvisade att risken för infektiösa komplikationer är signifikant förhöjd i över fem år postoperativt, och risken för tromboemboliska händelser (blodproppar) kvarstår i upp till tre år. Om alla patienter rutinmässigt skulle genomgå diagnostisk lymfkörtelutrymning innebär det att cirka 80 procent överbehandlas, med risk för betydande komplikationer helt utan onkologisk vinst.

För att identifiera patienter med regional spridning som har nytta av lymfkörtelutrymning, och samtidigt minimera överbehandling, utförs en minimalinvasiv kirurgisk stadieindelning med hjälp av dynamisk sentinel node-biopsi (DSNB). Metoden identifierar den eller de första lymfkörtlarna som tar emot lymfa från tumörområdet (portvaktskörtlarna) med hjälp av spårämne. DSNB har hög precision och färre biverkningar än diagnostisk lymfkörtelutrymning (ILND). Vid vår utvärdering av DSNB-metoden, vid ett av Sveriges två nationella centra för peniscancer, kunde resultaten bekräfta att metoden är känslig med låg risk för komplikationer och besparar över 80 procent av patienterna ett mycket mer omfattande kirurgiskt ingrepp (lymfkörtelutrymning) och dess biverkningar. Emellertid noterades en falskt negativ andel på 14,5 procent, vilket indikerar att det finns utrymme att ytterligare optimera den diagnostiska precisionen. Vidare framkom att risken för lokala komplikationer ökar i takt med antalet bortopererade lymfkörtlar, vilket starkt understryker vikten av strikt kirurgisk precision.

Humant papillomvirus (HPV) är en central orsak till sjukdomen och ligger bakom cirka 50 procent av alla peniscancerfall. Ett viktigt spår i avhandlingen har därför varit att undersöka sambandet mellan peniscancer och risken att senare i livet utveckla andra HPV-associerade maligniteter. Genom att analysera data från svenska kvalitets- och hälsoregister framkom att patienter med peniscancer löper en 2–3 gånger ökad risk att drabbas av cancer i munhåla, svalg och analkanal. Denna kunskap är av stor betydelse för utformningen av kliniska uppföljningsprogram och understryker vikten av det långsiktiga folkhälsoarbetet med könsneutral HPV-vaccination.

Ett centralt mål inom onkologin är att utveckla minimalinvasiva diagnostiska metoder för att mer träffsäkert identifiera patienter med tumörspridning i ett tidigt skede och samtidigt minimera behovet av kirurgiska ingrepp. I sökandet efter sådana metoder utforskade vi konceptet med så kallade flytande biopsier (*liquid biopsies*) genom att analysera 14 lösliga immuncheckpoint-proteiner i plasma från 284 peniscancerpatienter. Resultaten visade dock att en panel bestående av dessa proteiner saknar tillräcklig precision för att identifiera lymfkörtelmetastaser och därmed inte kan komplettera eller ersätta den nuvarande kirurgiska stadieindelningen med DSNB. Däremot observerades signifikant högre nivåer av fyra dämpande lösliga immuncheckpoint-proteiner (IDO, TIM-3, CD80 och CTLA-4) hos patienter med peniscancer jämfört med cancerfria kontroller. Detta tyder på att tumören sätter igång en immunhämning redan tidigt i sjukdomsförloppet, oberoende av tumörbörda eller förekomst av metastaser.

Sammantaget bidrar denna avhandling till en fördjupad förståelse för peniscancerns långsiktiga konsekvenser, både gällande kirurgins morbiditet och risken för andra HPV-associerade tumörer. Resultaten bekräftar värdet av DSNB för att minska kirurgisk sjuklighet, samtidigt som de belyser begränsningarna i dagens diagnostik och utforskar immunologiska biomarkörer som ett framtida steg mot en mer precis och individanpassad vård.

Grants

This work has been made possible by the kind support of the Lions Cancer Research Fund—Central Sweden and the Research Committee of Region Örebro County.

Acknowledgements

I would like to express my sincere gratitude to

Sabina Davidsson, my main supervisor, for your steadfast support, kindness, and boundless enthusiasm. Thank you for always being accessible and for your remarkably swift responses. I truly appreciate your open door; knowing I could approach you without hesitation, even when my questions were not strictly research-related, has meant the world to me. I cannot thank you enough for the profound impact you have had on my professional growth.

Peter Kirrander, my co-supervisor and exceptional clinical mentor. Thank you for teaching me the complexities of penile cancer, for your steady guidance in the operating theatre, and for opening the door to the world of medical science. Your remarkable expertise in both surgery and research is the very reason I was inspired to become a penile cancer specialist and embark on this doctoral journey in the first place.

Jessica Carlsson, my co-supervisor, for your invaluable guidance and for effortlessly navigating me through the complexities of statistics and lab work.

All my friends and colleagues at the Department of Urology. Thank you for your endless patience, your collegiality, and for making the department such a great place to work.

My co-workers from NPECR. Thank you for your excellent work with the registry and for your assistance with the statistical analyses for Papers I and II.

My loving parents, for your unconditional love, for always believing in me, and for supporting me throughout my entire life.

But most of all, my deepest gratitude goes to my family. To my wonderful children, **Hanna and Adam**, for brightening every single day. And especially to my incredible wife, **Andrea**, for your unwavering support over the years and your extraordinary patience during the countless times my mind was occupied with this thesis. I love you!

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49.
2. Douglawi A, Masterson TA. Penile cancer epidemiology and risk factors: a contemporary review. Current opinion in urology. 2019;29(2):145-9.
3. Moen CA, Gerdtsson A, Aagaard M, Hopland A, Kirrander P, Jakobsen JK. The past, present and future of penile cancer in the Nordic countries. Results from NORDCAN. BJUI Compass. 2025;6(7):e70041.
4. Nationellt_kvalitetsregister_for_peniscancer. Peniscancer - Nationell kvalitetsrapport för 2024. 2025.
5. Kirrander P, Sherif A, Friedrich B, Lambe M, Hakansson U, Steering Committee of the Swedish National Penile Cancer R. Swedish National Penile Cancer Register: incidence, tumour characteristics, management and survival. BJU Int. 2016;117(2):287-92.
6. Brouwer OR, Albersen M, Parnham A, Protzel C, Pettaway CA, Ayres B, et al. European Association of Urology-American Society of Clinical Oncology Collaborative Guideline on Penile Cancer: 2023 Update. Eur Urol. 2023;83(6):548-60.
7. Jakobsen JK, Sorensen CM, Krarup KP, Jensen JB. Quality of life, voiding and sexual function of penile cancer patients: DaPeCa-10-a cross-sectional questionnaire survey. BJUI Compass. 2022;3(5):354-62.
8. Skeppner E, Fugl-Meyer K. Dyadic Aspects of Sexual Well-Being in Men with Laser-Treated Penile Carcinoma. Sex Med. 2015;3(2):67-75.
9. Djajadiningrat RS, Graafland NM, van Werkhoven E, Meinhardt W, Bex A, van der Poel HG, et al. Contemporary management of

regional nodes in penile cancer-improvement of survival? J Urol. 2014;191(1):68-73.

10. Ferlay J EM, Lam F, Laversanne M, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. 2024.

11. Larke NL, Thomas SL, dos Santos Silva I, Weiss HA. Male circumcision and penile cancer: a systematic review and meta-analysis. Cancer Causes Control. 2011;22(8):1097-110.

12. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED, Jr., Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. N Engl J Med. 2011;364(5):401-11.

13. Drolet M, Benard E, Perez N, Brisson M, Group HPVVIS. Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: updated systematic review and meta-analysis. Lancet. 2019;394(10197):497-509.

14. Mannweiler S, Sygulla S, Winter E, Regauer S. Two major pathways of penile carcinogenesis: HPV-induced penile cancers overexpress p16ink4a, HPV-negative cancers associated with dermatoses express p53, but lack p16ink4a overexpression. J Am Acad Dermatol. 2013;69(1):73-81.

15. Nordanger IM, Beisland C, Thorkelsen TK, Honore A, Juliebo-Jones P, Bostad L, et al. The Prognostic Value of Human Papillomavirus Status in Penile Cancer: Outcomes From a Norwegian Cohort Study. Clin Genitourin Cancer. 2024;22(5):102127.

16. Velazquez EF, Chaux A, Cubilla AL. Histologic classification of penile intraepithelial neoplasia. Semin Diagn Pathol. 2012;29(2):96-102.

17. Bjartmar I, Gerdtsson A, Torbrand C, Kristiansen S. Risk of invasive penile cancer after treatment of penile intraepithelial neoplasia. BJU Int. 2025;136(1):73-81.

18. Hoekstra RJ, Trip EJ, Ten Kate FJ, Horenblas S, Lock MT. Penile intraepithelial neoplasia: Nomenclature, incidence and progression to malignancy in the Netherlands. Int J Urol. 2019;26(3):353-7.

19. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health. 2020;8(2):e180-e90.

20. Chesson HW, Dunne EF, Hariri S, Markowitz LE. The estimated lifetime probability of acquiring human papillomavirus in the United States. Sex Transm Dis. 2014;41(11):660-4.

21. Kirrander P, Kolaric A, Helenius G, Windahl T, Andren O, Stark JR, et al. Human papillomavirus prevalence, distribution and correlation to histopathological parameters in a large Swedish cohort of men with penile carcinoma. BJU Int. 2011;108(3):355-9.

22. Staerk MG, Munk C, Stilling C, Elversang J, Kjaer-Frifeldt S, Hjulskov SH, et al. Prevalence and time trends of human papillomavirus in penile cancer over 26 years-A comprehensive study of 1040 penile cancer cases from Denmark. Int J Cancer. 2026;158(9):2466-75.

23. Chaturvedi AK. Beyond cervical cancer: burden of other HPV-related cancers among men and women. J Adolesc Health. 2010;46(4 Suppl):S20-6.

24. Alemany L, Cubilla A, Halec G, Kasamatsu E, Quiros B, Masferrer E, et al. Role of Human Papillomavirus in Penile Carcinomas Worldwide. Eur Urol. 2016;69(5):953-61.

25. Olesen TB, Sand FL, Rasmussen CL, Albieri V, Toft BG, Norrild B, et al. Prevalence of human papillomavirus DNA and p16(INK4a) in penile cancer and penile intraepithelial neoplasia: a systematic review and meta-analysis. Lancet Oncol. 2019;20(1):145-58.

26. Chahoud J, Zacharias NM, Pham R, Qiao W, Guo M, Lu X, et al. Prognostic Significance of p16 and Its Relationship with Human Papillomavirus Status in Patients with Penile Squamous Cell

Carcinoma: Results of 5 Years Follow-Up. Cancers (Basel). 2022;14(24).

27. Longoni M, Fankhauser CD, Negri F, Salonia A, Basile G, Johnstone PAS, et al. Treatment strategies in human papillomavirus-related advanced penile cancer. Nat Rev Urol. 2025;22(7):427-38.

28. Moen CA, Falkenthal TE, Thorkelsen TK, Hopland A, Rio OE, Honore A, et al. Penile Cancers Attributed to Human Papillomavirus Are Associated with Improved Survival for Node-positive Patients. Findings from a Norwegian Cohort Study Spanning 50 Years. European urology oncology. 2024;7(4):778-85.

29. de Vries HM, Ottenhof SR, Horenblas S, van der Heijden MS, Jordanova ES. Defining the Tumor Microenvironment of Penile Cancer by Means of the Cancer Immunogram. European urology focus. 2019;5(5):718-21.

30. Glombik D, Oxelbark A, Sundqvist P, Carlsson J, Lambe M, Drevin L, et al. Risk of second HPV-associated cancers in men with penile cancer. Acta Oncol. 2021;60(5):667-71.

31. Suk R, Mahale P, Sonawane K, Sikora AG, Chhatwal J, Schmeler KM, et al. Trends in Risks for Second Primary Cancers Associated With Index Human Papillomavirus-Associated Cancers. JAMA Netw Open. 2018;1(5):e181999.

32. Philippou P, Shabbir M, Ralph DJ, Malone P, Nigam R, Freeman A, et al. Genital lichen sclerosus/balanitis xerotica obliterans in men with penile carcinoma: a critical analysis. BJU Int. 2013;111(6):970-6.

33. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860-7.

34. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.

35. Crusz SM, Balkwill FR. Inflammation and cancer: advances and new agents. Nat Rev Clin Oncol. 2015;12(10):584-96.

36. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39(1):1-10.

37. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-64.

38. Marin-Acevedo JA, Dholaria B, Soyano AE, Knutson KL, Chumsri S, Lou Y. Next generation of immune checkpoint therapy in cancer: new developments and challenges. J Hematol Oncol. 2018;11(1):39.

39. Marin-Acevedo JA, Kimbrough EO, Lou Y. Next generation of immune checkpoint inhibitors and beyond. J Hematol Oncol. 2021;14(1):45.

40. Udager AM, Liu TY, Skala SL, Magers MJ, McDaniel AS, Spratt DE, et al. Frequent PD-L1 expression in primary and metastatic penile squamous cell carcinoma: potential opportunities for immunotherapeutic approaches. Ann Oncol. 2016;27(9):1706-12.

41. Ottenhof SR, Djajadiningrat RS, de Jong J, Thygesen HH, Horenblas S, Jordanova ES. Expression of Programmed Death Ligand 1 in Penile Cancer is of Prognostic Value and Associated with HPV Status. J Urol. 2017;197(3 Pt 1):690-7.

42. Davidsson S, Carlsson J, Giunchi F, Harlow A, Kirrander P, Rider J, et al. PD-L1 Expression in Men with Penile Cancer and its Association with Clinical Outcomes. European urology oncology. 2019;2(2):214-21.

43. Tang Y, Hu X, Wu K, Li X. Immune landscape and immunotherapy for penile cancer. Frontiers in immunology. 2022;13:1055235.

44. Gu D, Ao X, Yang Y, Chen Z, Xu X. Soluble immune checkpoints in cancer: production, function and biological significance. Journal for immunotherapy of cancer. 2018;6(1):132.

45. Brierley JD GM, O'Sullivan B, Rous B, Van Eycken E, editors. TNM classification of malignant tumours. 9th ed.: Oxford: Wiley-Blackwell; 2025 May 2025.

46. Brierley J GM, Wittekind C, editors. TNM Classification of Malignant Tumours. 8th ed.: Oxford: Wiley-Blackwell; 2016.

47. Horenblas S, Jansen L, Meinhardt W, Hoefnagel CA, de Jong D, Nieweg OE. Detection of occult metastasis in squamous cell carcinoma of the penis using a dynamic sentinel node procedure. J Urol. 2000;163(1):100-4.

48. Moen CA, Jakobsen JK. Sentinel Node Mapping in Penile Cancer: An Update on Methods and Pitfalls. Semin Nucl Med. 2026;56(2):221-8.

49. Kroon BK, Horenblas S, Lont AP, Tanis PJ, Gallee MP, Nieweg OE. Patients with penile carcinoma benefit from immediate resection of clinically occult lymph node metastases. J Urol. 2005;173(3):816-9.

50. Graafland NM, Lam W, Leijte JA, Yap T, Gallee MP, Corbishley C, et al. Prognostic factors for occult inguinal lymph node involvement in penile carcinoma and assessment of the high-risk EAU subgroup: a two-institution analysis of 342 clinically node-negative patients. Eur Urol. 2010;58(5):742-7.

51. Stuiver MM, Djajadiningrat RS, Graafland NM, Vincent AD, Lucas C, Horenblas S. Early wound complications after inguinal lymphadenectomy in penile cancer: a historical cohort study and risk-factor analysis. Eur Urol. 2013;64(3):486-92.

52. Gopman JM, Djajadiningrat RS, Baumgarten AS, Espiritu PN, Horenblas S, Zhu Y, et al. Predicting postoperative complications of inguinal lymph node dissection for penile cancer in an international multicentre cohort. BJU Int. 2015;116(2):196-201.

53. Koifman L, Hampl D, Koifman N, Vides AJ, Ornellas AA. Radical open inguinal lymphadenectomy for penile carcinoma: surgical technique, early complications and late outcomes. J Urol. 2013;190(6):2086-92.

54. Leijte JA, Kroon BK, Valdes Olmos RA, Nieweg OE, Horenblas S. Reliability and safety of current dynamic sentinel node biopsy for penile carcinoma. Eur Urol. 2007;52(1):170-7.

55. Fankhauser CD, de Vries HM, Roussel E, Jakobsen JK, Issa A, Lee EWC, et al. Lymphovascular and perineural invasion are risk factors for inguinal lymph node metastases in men with T1G2 penile cancer. J Cancer Res Clin Oncol. 2022;148(9):2231-4.

56. Gerdtsson A, Abedi E, Baseckas G, Brorson H, Dorofte L, Fall S, et al. The Swedish national guidelines on penile cancer. Scand J Urol. 2025;60:189-94.

57. Sadeghi R, Gholami H, Zakavi SR, Kakhki VR, Tabasi KT, Horenblas S. Accuracy of sentinel lymph node biopsy for inguinal lymph node staging of penile squamous cell carcinoma: systematic review and meta-analysis of the literature. J Urol. 2012;187(1):25-31.

58. Wever L, de Vries HM, Dell'Oglio P, van der Poel HG, Donswijk ML, Sikorska K, et al. Incidence and risk factor analysis of complications after sentinel node biopsy for penile cancer. BJU Int. 2022;130(4):486-95.

59. Jakobsen JK, Krarup KP, Sommer P, Nerstrom H, Bakholdt V, Sorensen JA, et al. DaPeCa-1: diagnostic accuracy of sentinel lymph node biopsy in 222 patients with penile cancer at four tertiary referral centres - a national study from Denmark. BJU Int. 2016;117(2):235-43.

60. Lam W, Alnajjar HM, La-Touche S, Perry M, Sharma D, Corbishley C, et al. Dynamic sentinel lymph node biopsy in patients with invasive squamous cell carcinoma of the penis: a prospective study of the long-term outcome of 500 inguinal basins assessed at a single institution. Eur Urol. 2013;63(4):657-63.

61. Kirrander P, Andrén O, Windahl T. Dynamic sentinel node biopsy in penile cancer: initial experiences at a Swedish referral centre. BJU Int. 2013;111(3 Pt B):E48-53.

62. Torbrand C, Warnolf A, Glombik D, Davidsson S, Carlsson J, Baseckas G, et al. Sentinel Node Identification with Hybrid Tracer-guided and Conventional Dynamic Sentinel Node Biopsy in Penile Cancer: A Prospective Study in 130 Patients from the

Two National Referral Centres in Sweden. European urology oncology. 2022;5(6):704-11.

63. Kroon BK, Horenblas S, Deurloo EE, Nieweg OE, Teertstra HJ. Ultrasonography-guided fine-needle aspiration cytology before sentinel node biopsy in patients with penile carcinoma. BJU Int. 2005;95(4):517-21.

64. Lee EWC, Issa A, Oliveira P, Lau M, Sangar V, Parnham A, et al. High diagnostic accuracy of inguinal ultrasonography and fine-needle aspiration followed by dynamic sentinel lymph node biopsy in men with impalpable and palpable inguinal lymph nodes. BJU Int. 2022;130(3):331-6.

65. Kamel MH, Bissada N, Warford R, Farias J, Davis R. Organ Sparing Surgery for Penile Cancer: A Systematic Review. J Urol. 2017;198(4):770-9.

66. Hakansson U, Kirrander P, Uvelius B, Baseckas G, Torbrand C. Organ-sparing reconstructive surgery in penile cancer: initial experiences at two Swedish referral centres. Scand J Urol. 2015;49(2):149-54.

67. Fallara G, Basile G, Poterek A, Tozzi M, Pang KH, Cakir OO, et al. Oncological and Functional Outcomes of Penile Shaft Sparing Surgery for Localised Penile Cancer: A Systematic Review. European urology focus. 2025;11(1):46-61.

68. Lindner AK, Schachtner G, Steiner E, Kroiss A, Uprimny C, Steinkohl F, et al. Organ-sparing surgery of penile cancer: higher rate of local recurrence yet no impact on overall survival. World J Urol. 2020;38(2):417-24.

69. Lasorsa F, Bignante G, Orsini A, Rossetti SS, Marchioni M, Porpiglia F, et al. Follow Up Care After Penile Sparing Surgery for Penile Cancer: Current Perspectives. Res Rep Urol. 2024;16:225-33.

70. Minhas S, Kayes O, Hegarty P, Kumar P, Freeman A, Ralph D. What surgical resection margins are required to achieve oncological control in men with primary penile cancer? BJU Int. 2005;96(7):1040-3.

71. Philippou P, Shabbir M, Malone P, Nigam R, Muneer A, Ralph DJ, et al. Conservative surgery for squamous cell carcinoma of the penis: resection margins and long-term oncological control. J Urol. 2012;188(3):803-8.

72. Sri D, Sujenthiran A, Lam W, Minter J, Tinwell BE, Corbishley CM, et al. A study into the association between local recurrence rates and surgical resection margins in organ-sparing surgery for penile squamous cell cancer. BJU Int. 2018;122(4):576-82.

73. Protzel C, Alcaraz A, Horenblas S, Pizzocaro G, Zlotta A, Hakenberg OW. Lymphadenectomy in the surgical management of penile cancer. Eur Urol. 2009;55(5):1075-88.

74. Lee SW, Kim SJ. Diagnostic Performance of 18F-FDG PET/CT for Lymph Node Staging in Penile Cancer. Clin Nucl Med. 2022;47(5):402-8.

75. Djajadiningrat RS, van Werkhoven E, Horenblas S. Prophylactic pelvic lymph node dissection in patients with penile cancer. J Urol. 2015;193(6):1976-80.

76. Liu JY, Li YH, Zhang ZL, Yao K, Ye YL, Xie D, et al. The risk factors for the presence of pelvic lymph node metastasis in penile squamous cell carcinoma patients with inguinal lymph node dissection. World J Urol. 2013;31(6):1519-24.

77. Yao K, Chen Y, Ye Y, Wu Z, Chen D, Han H, et al. Lymph Node Mapping in Patients with Penile Cancer Undergoing Pelvic Lymph Node Dissection. J Urol. 2021;205(1):145-51.

78. Sood A, Rudzinski JK, Spiess PE, Pettaway CA. The Acute Complications After Surgery for Penile Carcinoma and Strategies for Their Management: A Systematic Review of the Literature. Semin Oncol Nurs. 2022;38(3):151285.

79. Glombik D, Davidsson S, Sandin F, Lambe M, Carlsson J, Sundqvist P, et al. Penile cancer: long-term infectious and thromboembolic complications following lymph node dissection - a population-based study (Sweden). Acta Oncol. 2023;62(5):458-64.

80. Juliebo-Jones P, Nordanger IM, Beisland C, Thorkelsen TK, Honore A, Moen CA. Early and Late Complications Associated with Penile Cancer Surgery and the Impact of Human Papillomavirus Status: Findings from a Retrospective Norwegian Cohort Study. Eur Urol Open Sci. 2025;72:29-35.

81. Heyns CF, Fleshner N, Sangar V, Schlenker B, Yuvaraja TB, van Poppel H. Management of the lymph nodes in penile cancer. Urology. 2010;76(2 Suppl 1):S43-57.

82. Bouchot O, Rigaud J, Maillet F, Hetet JF, Karam G. Morbidity of inguinal lymphadenectomy for invasive penile carcinoma. Eur Urol. 2004;45(6):761-5; discussion 5-6.

83. Perdona S, Autorino R, De Sio M, Di Lorenzo G, Gallo L, Damiano R, et al. Dynamic sentinel node biopsy in clinically node-negative penile cancer versus radical inguinal lymphadenectomy: a comparative study. Urology. 2005;66(6):1282-6.

84. Eskenazi F, Medina LG, Soto Suarez R, Fumero L, Lusinchi Delfino AC, Patel K, et al. Optimizing Inguinal Lymph Node Dissection for Penile Cancer: A Pathway to Improve Outcomes and Complications—A Narrative Review. Complications. 2025;2(3):20.

85. Spiess PE, Hernandez MS, Pettaway CA. Contemporary inguinal lymph node dissection: minimizing complications. World J Urol. 2009;27(2):205-12.

86. Koifman L, Hampl D, Ginsberg M, Castro RB, Koifman N, Ornellas P, et al. The role of primary inguinal surgical debulking for locally advanced penile cancer followed by reconstruction with myocutaneous flap. Int Braz J Urol. 2021;47(6):1162-75.

87. Sachdeva A, McGuinness L, Zapala L, Greco I, Garcia-Perdomo HA, Kailavasan M, et al. Management of Lymph Node-positive Penile Cancer: A Systematic Review. Eur Urol. 2024;85(3):257-73.

88. Ulvskog E, Persson EK, Kirrander P, Fall K, Ahlgren J. Nationwide Data Support Centralised Decision-making in Penile

Cancer Care: A Before-and-After Study on Guideline Adherence and Disease-specific survival for Patients with an Indication for Perioperative Oncological Treatment. Eur Urol Open Sci. 2023;51:70-7.

89. Yi XY, Cao DH, You PH, Xiong XY, Zheng XN, Peng G, et al. Neoadjuvant chemotherapy for patients with locally advanced penile cancer: an updated evidence. Asian J Androl. 2022;24(2):180-5.

90. Patel KN, Salunke A, Bakshi G, Jayaprakash D, Pandya SJ. Robotic-Assisted Video-Endoscopic Inguinal Lymphadenectomy (RAVEIL) and Video-Endoscopic Inguinal Lymphadenectomy (VEIL) versus Open Inguinal Lymph-Node Dissection (OILND) in carcinoma of penis: Comparison of perioperative outcomes, complications and oncological outcomes. A systematic review and meta-analysis. Urol Oncol. 2022;40(3):112 e11- e22.

91. Vreeburg MTA, de Vries HM, van der Noort V, Horenblas S, van Rhijn BWG, Hendricksen K, et al. Penile cancer care in the Netherlands: increased incidence, centralisation, and improved survival. BJU Int. 2024;133(5):596-603.

92. Jakobsen JK, Pettaway CA, Ayres B. Centralization and Equitable Care in Rare Urogenital Malignancies: The Case for Penile Cancer. European urology focus. 2021;7(5):924-8.

93. Yan S, Longoni M, Basile G, Fankhauser CD, Nardo ND, Sanchez R, et al. Current practices and variability in dynamic sentinel node biopsy for penile cancer: A survey of European Referral Centers. Urol Oncol. 2025;43(11):665 e11- e16.

94. Medina LG, Hatoum F, Paesano N, Zemp L, Grass D, Morse B, et al. Multidisciplinary Care of Patients With Penile Cancer. Am Soc Clin Oncol Educ Book. 2026;46(2):e516022.

95. Warnolf A, Glombik D, Sandin F, Lambe M, Baseckas G, Gerdtsson A, et al. Evaluation of data quality in the Swedish National Penile Cancer Register. Scand J Urol. 2024;59:162-8.

96. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009;48(1):27-33.

97. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011;11:450.

98. Wettermark B, Hammar N, Fored CM, Leimanis A, Otterblad Olausson P, Bergman U, et al. The new Swedish Prescribed Drug Register--opportunities for pharmacoepidemiological research and experience from the first six months. Pharmacoepidemiology and drug safety. 2007;16(7):726-35.

99. (Socialstyrelsen) TNBoHaW. Statistical Database on Cancer 2025 [Available from: https://sdb.socialstyrelsen.se/if_can/val_eng.aspx.

100. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240(2):205-13.

101. Mitropoulos D, Artibani W, Biyani CS, Bjerggaard Jensen J, Roupret M, Truss M. Validation of the Clavien-Dindo Grading System in Urology by the European Association of Urology Guidelines Ad Hoc Panel. European urology focus. 2018;4(4):608-13.

102. Scott-Wittenborn N, Fakhry C. Epidemiology of HPV Related Malignancies. Semin Radiat Oncol. 2021;31(4):286-96.

103. Nelson RA, Lai LL. Elevated risk of human papillomavirus-related second cancers in survivors of anal canal cancer. Cancer. 2017;123(20):4013-21.

104. Neumann F, Jegu J, Mougin C, Pretet JL, Guizard AV, Lapotre-Ledoux B, et al. Risk of second primary cancer after a first potentially-human papillomavirus-related cancer: A population-based study. Prev Med. 2016;90:52-8.

105. Gilbert DC, Wakeham K, Langley RE, Vale CL. Increased risk of second cancers at sites associated with HPV after a prior HPV-associated malignancy, a systematic review and meta-analysis. Br J Cancer. 2019;120(2):256-68.

106. Burchell AN, Winer RL, de Sanjose S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. Vaccine. 2006;24 Suppl 3:S3/52-61.

107. Jeanne-Julien A, Bouchot O, De Vergie S, Branchereau J, Perrouin-Verbe MA, Rigaud J. Morbidity and risk factors for complications of inguinal lymph node dissection in penile cancer. World J Urol. 2023;41(1):109-18.

108. Qureshi W, Ali Z, Amjad W, Alirhayim Z, Farooq H, Qadir S, et al. Venous Thromboembolism in Cancer: An Update of Treatment and Prevention in the Era of Newer Anticoagulants. Front Cardiovasc Med. 2016;3:24.

109. Blom JW, Doggen CJ, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. JAMA. 2005;293(6):715-22.

110. Sharma P, Zargar H, Spiess PE. Surgical Advances in Inguinal Lymph Node Dissection: Optimizing Treatment Outcomes. Urol Clin North Am. 2016;43(4):457-68.

111. Suartz CV, de Lima RD, Abud LR, Brito PHS, Galhardo KA, Talizin TB, et al. Comparing open and video endoscopic lymphadenectomy for penile cancer: a systematic review and meta-analysis of prospective studies. BJU Int. 2025;135(4):567-76.

112. Mueller-Lisse UG, Scher B, Scherr MK, Seitz M. Functional imaging in penile cancer: PET/computed tomography, MRI, and sentinel lymph node biopsy. Current opinion in urology. 2008;18(1):105-10.

113. Dimopoulos P, Christopoulos P, Shilito S, Gall Z, Murby B, Ashworth D, et al. Dynamic sentinel lymph node biopsy for penile cancer: a comparison between 1- and 2-day protocols. BJU Int. 2016;117(6):890-6.

114. Fallara G, Pozzi E, Onur Cakir O, Tandogdu Z, Castiglione F, Salonia A, et al. Diagnostic Accuracy of Dynamic Sentinel Lymph Node Biopsy for Penile Cancer: A Systematic Review and Meta-analysis. European urology focus. 2023;9(3):500-12.

115. Wang Q, Zhang J, Tu H, Liang D, Chang DW, Ye Y, et al. Soluble immune checkpoint-related proteins as predictors of tumor recurrence, survival, and T cell phenotypes in clear cell renal cell carcinoma patients. Journal for immunotherapy of cancer. 2019;7(1):334.

116. Bian B, Fanale D, Dusetti N, Roque J, Pastor S, Chretien AS, et al. Prognostic significance of circulating PD-1, PD-L1, pan-BTN3As, BTN3A1 and BTLA in patients with pancreatic adenocarcinoma. Oncoimmunology. 2019;8(4):e1561120.

117. Li B, Wang Q, Luo Y, Wang S, Pan S, Zhao W, et al. Peripheral Soluble Immune Checkpoint-Related Proteins Were Associated with Survival and Treatment Efficacy of Osteosarcoma Patients, a Cohort Study. Cancers (Basel). 2024;16(9).

118. Ulvskog E, Kirrander P, Persson EK, Lillsunde-Larsson G, Dorofte L, Franceschini T, et al. Expression of PD-L1, TIGIT, and CD155, and Human Papillomavirus Status in Patients with Advanced Penile Cancer. Eur Urol Open Sci. 2025;79:102-10.

119. Dorofte L, Davidsson S, Carlsson J, Larsson GL, Karlsson MG. New histological risk grading system for prediction of lymph node metastasis in patients with penile cancer. Virchows Arch. 2025;486(4):759-67.

120. Pekarek L, Ortega MA, Fraile-Martinez O, Garcia-Montero C, Casanova C, Saez MA, et al. Clinical and Novel Biomarkers in Penile Carcinoma: A Prospective Review. J Pers Med. 2022;12(9).

121. Patel KR, Moon SC, Shumaker LA, Patel S, Galgano SJ, Ferguson JE, 3rd, et al. Utility of Tumor-Informed Circulating Tumor DNA for Monitoring Treatment Response in Nonmetastatic, Locally Advanced Penile and Primary Urethral Cancers. JCO Precis Oncol. 2025;9:e2500045.

122. Gerke MB, Jansen CS, Bilen MA. Circulating Tumor DNA in Genitourinary Cancers: Detection, Prognostics, and Therapeutic Implications. Cancers (Basel). 2024;16(12).

123. Wolff E, Schollin Ask L, Larsson S. Cost-effectiveness analyses of catch-up vaccination against human papillomavirus (HPV) related cancers of boys and young men in Sweden. Vaccine. 2026;72:128077.

124. Dykens JA, Peterson CE, Holt HK, Harper DM. Gender neutral HPV vaccination programs: Reconsidering policies to expand cancer prevention globally. Front Public Health. 2023;11:1067299.