Diagnostic Tools in the Evaluation and Management of Penile Cancer

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Pranav Sharma, Mariela R. Pow-Sang, and Julio M. Pow-Sang

Introduction

Penile cancer is rare in the industrialized countries of North America and Europe, representing only 0.4–0.6 % of all malignancies in men [1]. In the third-world nations of South America, Africa, and Asia, however, poor hygiene, low socioeconomic status, low circumcision rates, and high sexual promiscuity result in a much higher incidence of 1–2 % [2, 3].

Squamous cell carcinoma (SCC) represents the far majority of cases with verrucous, warty, and papillary subtypes having a more favorable prognosis and basaloid, sarcomatoid, and adenosquamous histologies having a less favorable prognosis with early metastatic spread [4].

The presence of human papillomavirus (HPV) DNA has also been detected in approximately 60–80 % of penile cancer tumors [5]. The most common subtypes of HPV are HPV-16 and -18 with the development of cancer mediated through oncogenes E6 and E7 and their downstream effects on tumor suppresser genes such as

P. Sharma, M.D. • J.M. Pow-Sang, M.D. (✉)
Department of Genitourinary Oncology, Moffitt Cancer Center,
12902 Magnolia Drive, Tampa, FL 33612, USA
e-mail: pranav.sharma@moffitt.org; julio.powsang@moffitt.org

M.R. Pow-Sang, M.D.
Department of Urology, Instituto Nacional de Enfermedades Neoplásicas,
Av. Angamos # 2520-Surquillo, Lima, Peru
e-mail: mrpowsang@hotmail.com

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p53 and Rb1 [3]. The prognostic role of HPV in penile cancer is not clearly known with some studies showing favorable prognosis in HPV-positive penile tumors, while others reporting no appreciable effect on cancer outcomes in the presence of HPV infection [6, 7]. Expression of HPV, however, may predict not only disease prognosis but also treatment response to surgery, radiation, and systemic therapy, which has been shown to be the case in other SCC tumor phenotypes particularly of the head and neck [8].

Penile carcinoma can be effectively cured in up to 80% of patients if treated appropriately at an early stage with aggressive management of the inguinal region in high-risk cases even in the absence of clinical disease [9]. Pathologic stage and grade of the primary penile tumor drives survival in addition to the extent of subsequent loco-regional lymph node (LN) spread [10].

Treatment paradigms have shifted to emphasize the increase utilization of penile sparing approaches for treatment of the primary tumor while identifying patients who will most likely benefit from inguinal and/or pelvic lymphadenectomy as well as the use of neoadjuvant chemotherapy in bulky nodal disease [11]. Novel diagnostic tools to more effectively identify these patients are essential to better customize treatment options for penile cancer and minimize its associated morbidity.

Novel Image-Based Diagnostic Tools

Evaluation and Management of the Primary Penile Lesion

Surgical resection of the primary penile tumor should result in complete removal of the cancerous lesion with negative surgical margins to minimize the risk of recurrence [12]. Treatment of the primary penile tumor can be curative, but it can also be devastating to a patient's quality of life (QOL) and mental well-being. Partial or complete penile amputation is associated with significant psychological morbidity, voiding, and sexual dysfunction [13].

Primary treatment of the penile tumor has historically involved radical or partial penectomy with a 2-cm margin for oncologic efficacy, but the 2-cm margin is a historical value with little scientific evidence to support it [14]. A recent literature review on penile-sparing surgery (PSS) showed that cancer-specific survival (CSS) is similar for penile sparing and ablative techniques for low-stage disease while providing better functional and cosmetic outcomes [15]. There are no randomized controlled studies comparing primary tumor treatments, and thus the level of evidence is based on retrospective analyses and small cohorts. Despite lack of level one evidence, however, penile sparing strategies should be employed for low-stage distal penile tumors whenever possible in order to preserve anatomical and sexual function [16].

Magnetic resonance imaging (MRI) may be useful in deciphering which patients may be appropriate for PSS [17]. This imaging modality may accurately predict corpora cavernosa or corpora spongiosum invasion as well as proximal extent of tumor involvement on the penile shaft or glans (Fig. 2.1) [18]. MRI has also been

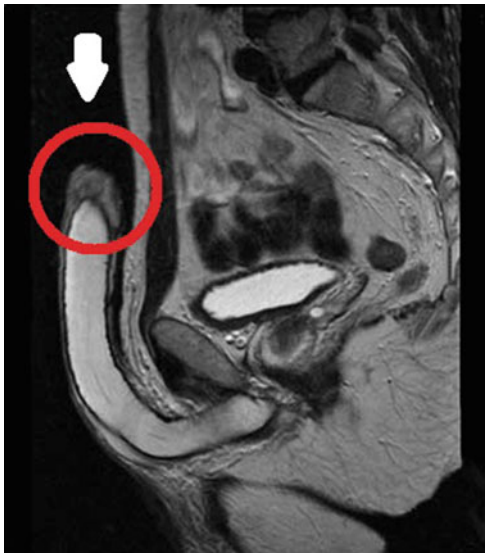

Fig. 2.1 Magnetic resonance imaging of a penile carcinoma of the glans with the proximal extent of tumor seen on the penile shaft

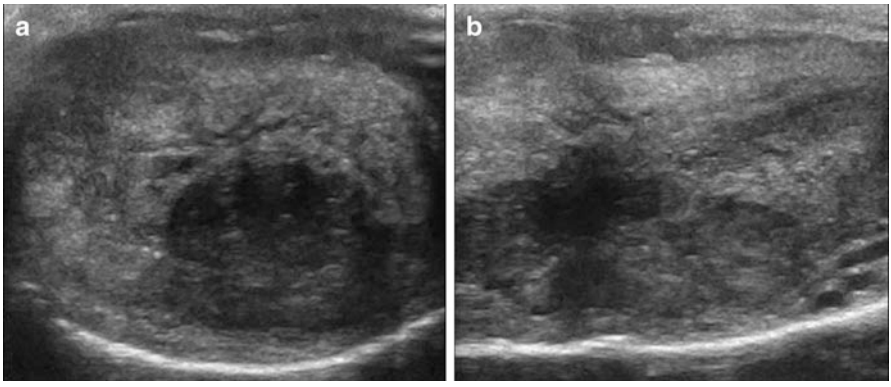

Fig. 2.2 Heterogeneous penile tumor visualized transversely (**a**) and longitudinally (**b**) on penile Doppler ultrasound

shown to be highly accurate in the local staging of penile cancer with stage-specific sensitivities and specificities of 85 and 83 % (pT1), 75 and 89 % (pT2), and 88 and 98 % (pT3) [19]. MRI, therefore, can accurately predict corpora cavernosa invasion in all cases of pathologically proven disease in order to maximize penile preservation when appropriate [20].

There is recent evidence to suggest that penile Doppler ultrasound (US) may be equivalent to MRI in the preoperative diagnostic evaluation of patients with penile SCC (Fig. 2.2) [21, 22]. In a prospective study of 200 patients presenting with a clinical diagnosis of penile SCC, penile Doppler US versus MRI accuracy in predicting primary tumor stage after surgery was 96.5 % versus 90.5 %, precision was

92.6 % versus 96 %, sensitivity was 96.9 % versus 73.8 %, and specificity was 96.2 % versus 98.5 %, respectively [23]. The authors concluded, therefore, that penile Doppler US had a statistically similar outcome in detecting tumor infiltration of the corpora cavernosa compared to MRI, and it could be used as a less expensive tool to drive surgical strategy in patient with a diagnosis of penile SCC.

Evaluation and Management of Loco-Regional Metastatic Lymphatic Spread

Multiple different imaging modalities have been explored to more accurately predict metastatic lymphatic spread for high-risk penile tumors. The value of 18F-fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET-CT) has recently come into the spotlight in the clinical staging of penile cancer due to its increased utilization and value in other aspects of oncology [24]. Scher et al. [25] initially demonstrated the diagnostic value of 18F-FDG PET-CT in 13 patients with suspected penile cancer or suspected recurrent disease and correlated this with histopathological findings obtained at the time of biopsy or during surgery. The sensitivity and specificity for PET-CT imaging to detect malignancy in the primary penile lesion was 75 % and 75 %, respectively, but it was 80 % and 100 % for the detection of malignancy in the LNs (sensitivity: 89 % for superficial inguinal LNs, 100 % for deep and pelvic LNs) (Fig. 2.3).

Leijte et al. [26] subsequently evaluated 18F-FDG PET-CT to detect occult inguinal metastasis in patients with clinically node negative (cN0) penile carcinoma. Only one of the five tumor-positive cN0 groins was correctly predicted by PET-CT although 34 of 37 negative groins were appropriately ruled out by preoperative imaging (specificity, 92 %). This same group also evaluated the diagnostic accuracy of 18F-FDG PET-CT to detect pelvic nodal involvement in 18 patients with unilateral or bilateral tumor-positive inguinal nodes on cytological assessment. Ten of 11 tumor-positive pelvic nodal basins were correctly predicted by PET-CT scan (sensitivity 91 %) as were all 17 tumor-negative pelvic nodal basins (specificity

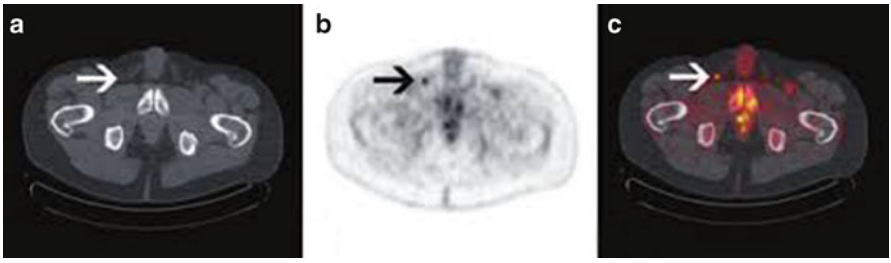

Fig. 2.3 Suspicious inguinal node detected on 18F-fluorodeoxyglucose positron emission tomography-computed tomography in a high-risk cancer. (**a**) CT image; (**b, c**) Contrast image

100 %). Four of five patients with positive distant metastasis on PET-CT had pathologically confirmed M1 disease (sensitivity 75 %). The authors, therefore, concluded that PET-CT may be useful in the routine clinical staging for inguinal node positive patients to detect further disease progression [27].

More recently, Souillac et al. [28] evaluated 22 patients with invasive SCC of the penis and negative groins (cN0) with 18F-FDG PET-CT to assess inguinal LN status. Eight patients with clinically node positive (cN+) groins were also assessed separately. Of 44 cN0 groins, PET-CT had a 75 % sensitivity and 87.5 % specificity, but it had 100 % sensitivity and 100 % specificity in cN+ groins. Schlenker et al. [29] also showed an 88.2 % sensitivity rate and a 98.1 % specificity rate for PET-CT in 70 inguinal groins (35 patients) with invasive penile carcinoma staged with this modality. All missed groin metastasis in both of the above studies were less than 1 cm in size. These results demonstrate that PET-CT may be useful in confirming inguinal LN invasion as well as detecting subclinical inguinal LN invasion in a large majority of cases although its ability to detect micro-metastasis has come into question due to higher false negative rates reported by some centers [30]. Close follow-up in these patients, therefore, is still recommended and is imperative to avoid subpar oncological outcomes.

A comprehensive systematic review and meta-analysis of the literature showed a pooled sensitivity and specificity of 80.9 % and 92.4 %, respectively, for 18F-FDG PET-CT in the accuracy of inguinal LN staging for penile SCC [31]. The pooled sensitivity was 96.4 % for cN+ patients and 56.5 % for cN0 patients. The authors, therefore, concluded that routine use of PET-CT is not justified, but patients with clinically palpable LNs may benefit due to the higher sensitivity of this technology in this subgroup of patients. Future clinical trials, however, comparing PET-CT to standard clinical assessment (i.e. physical examination) are necessary to truly elucidate the benefits that this additional imaging can provide in terms of early detection of occult metastatic disease in the groin or pelvis, minimizing patient morbidity from unnecessary treatments, and possibly improving survival-related outcomes.

Magnetic resonance imaging (MRI) and MRI-PET are additional imaging techniques that may be useful in both local and LN staging for penile cancer (Fig. 2.4). Novel magnetic resonance (MR) imaging techniques such as lymphotropic nanoparticle-enhanced MR imaging may help identify metastatic LN disease [32]. Currently, a clinical trial is being conducted and is recruiting patients in the United Kingdom to establish the effectiveness of MRI-PET compared to dynamic sentinel node biopsy (DSNB) and ultrasound-guided biopsy in detecting the presence of metastatic disease in the LNs of patients with penile cancer. If MRI-PET is effective in detecting LN involvement in patients with locally advanced penile cancer, it could potentially replace these more invasive procedures.

Additionally, while DSNB has traditionally been performed with radiotracer ^{99m}Tc-nanocolloid, new literature suggests the possible use of a fluorescent dye called indocyanine green (ICG) with similar effectiveness. Markuszewski et al. [33] recently reported on a small prospective study of 14 patients who underwent

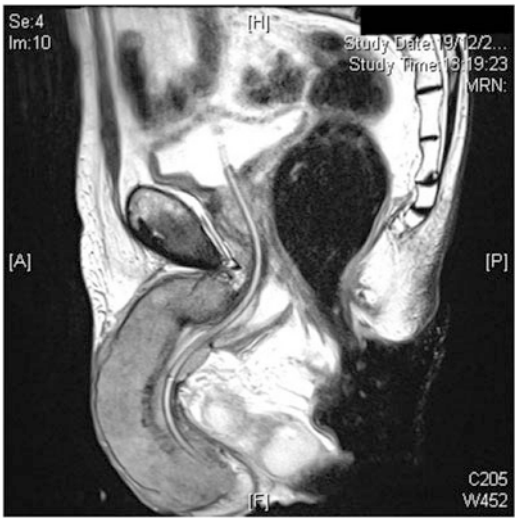

Fig. 2.4 Locally advanced penile carcinoma seen on magnetic resonance imaging

injection of both ^{99m}Tc-nanocolloid and ICG at the primary penile tumor site just before DSNB. Sentinel LNs (SLNs) were localized intraoperatively using the gamma-ray detection probe for radiocolloid and near-infrared fluorescence (NIRF) camera for ICG. Percutaneously, LNs were identified in all 14 patients using the gamma probe and in 10 patients using the NIRF camera. After skin incision, fluorescent nodes were observed using the NIRF camera in the remaining four patients. The intraoperative examination led to the identification of 32 total SLNs using technetium and ICG and additionally three more nodes visible only using ICG. Of the 35 SLNs, 30 were negative and 4 were positive for metastasis. Brouwer et al. [34] also reported on a hybrid radioactive and fluorescent tracer for DSNB in penile cancer as a potential replacement for blue dye (Fig. 2.5). Sixty-five patients with penile SCC underwent peritumor injection of a combination ICG-(99m) Tc-nanocolloid tracer prior to surgery followed by patent blue dye and/or NIRF imaging. Fluorescence imaging enabled visualization of 96.8 % of SLNs, while only 55.7 % were stained by blue dye ($P < 0.01$), suggesting a hybrid radioactive and fluorescent ICG-(99m)Tc-nanocolloid tracer can improve optical SLN detection compared with blue dye.

Other future directions for novel imaging strategies in penile cancer include molecular imaging with ferrous nanoparticles or alternative nontoxic drug delivery systems. These agents can potential identify and label penile cancer cells and enhance MRI visualization of micro-metastatic disease not visible with traditional imaging [35]. Various applications using targeted iron oxide nanoparticles have been evaluated in vitro and in animal experiments for the labeling of mesenchymal stem cells and dendritic cells [36]. Future studies, however, will be needed to determine their utility in vivo in penile cancer patients.

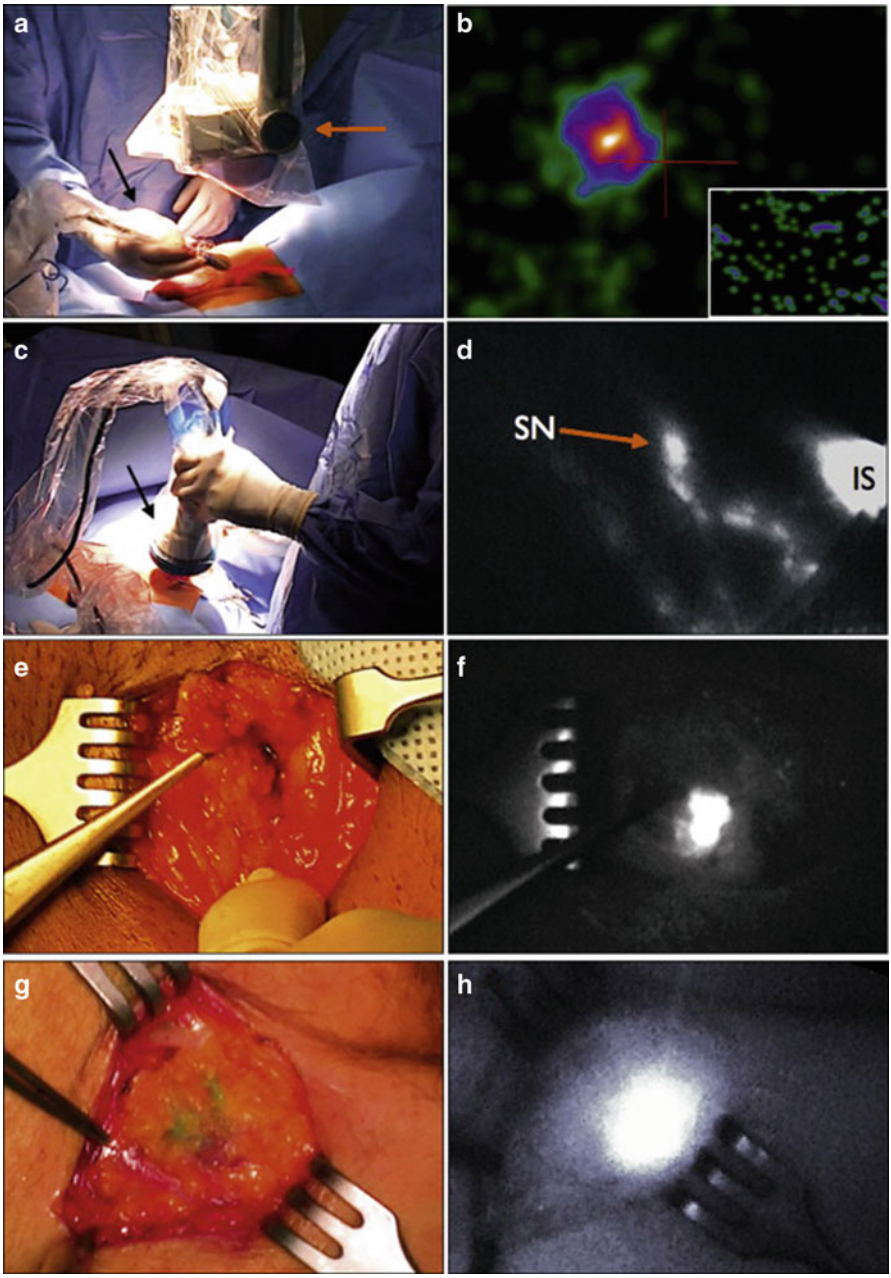

Fig. 2.5 A hybrid radioactive and fluorescent tracer technique using indocyanine green for dynamic sentinel node biopsy in penile cancer as a potential replacement for blue dye. (**a, c, e,** and **g**) Probe position at lymph node area. (**b, d, f** and **h**) Images showing sentinel node

Penile Cancer Biomarkers

Advancement in the techniques for molecular genomics has made biomarkers an increasingly important aspect of a clinician's diagnostic and predictive tools with regard to penile tumor metastasis and disease recurrence. A list of several biomarkers studied in penile carcinoma is summarized in Table 2.1.

Despite initial promising results, the evidence supporting the routine use of biomarkers in the diagnosis and management of penile cancer is still not well established enough to consider their inclusion in cancer guidelines [37]. Data are still controversial regarding the ability of biomarkers to predict the presence of occult LN metastasis. There is a need for large prospective studies to ascertain the clinical utility of biomarkers, but several candidates have been shown to be potential candidates for future investigation.

p53

Tumor protein p53 is a tumor suppressor gene that plays a role in apoptosis, genomic stability, and inhibition of angiogenesis. It can activate DNA repair proteins when DNA has sustained damage and can arrest growth by holding the cell cycle at the G1/S regulation point on DNA damage recognition. The International Cancer Genome Consortium has established that the p53 gene is the most frequently mutated gene (>50 %) in human cancer, indicating that the p53 gene plays a crucial role in preventing cancer formation [38].

Expression of p53 has been evaluated in several studies with regard to prognosis in penile carcinoma. Lopes et al. initially studied 82 patients with penile carcinoma who underwent amputation and bilateral lymphadenectomy to evaluate the prognostic value of immunohistochemical p53 staining in the primary penile tumor [39]. Immunoreactivity of p53 was studied with other clinical and pathological variables, including patient age, stage, histological grade, tumor thickness, lymphatic and venous embolization, corpora cavernosa, corpus spongiosum and urethral infiltration, and HPV status. The association of p53 with LN metastasis, survival, and risk of death was determined as the primary endpoints.

Nuclear accumulation of p53 was detected in 34 of 82 samples in the study (41.5 %) [39]. Clinical nodal stage ($P=0.045$), lymphatic ($P<0.001$) and venous ($P=0.04$) embolization by neoplastic cells, p53 positivity ($P=0.012$), and p53 grade ($P=0.004$) were all significantly associated with LN metastasis. Multivariate analysis revealed that only lymphatic embolization (relative risk [RR], 9.4; 95 % CI, 2.8–31.6) and p53 positivity (RR, 4.8; 95 % CI, 1.6–14.9) were independent factors for LN metastasis. Patients with negative p53 had significantly better 5- and 10-year overall survival (OS) than those in whom tumors stained positive for p53 (64.5 % and 54.6 % vs. 30.2 % and 26.4 %, respectively; $P=0.009$). When tumors were p53 positive and HPV DNA positive, OS was worse. Multivariate analysis, however, revealed that only age (RR, 2.9; 95 % CI, 1.6–5.1) and LN metastasis (RR, 3.2; 95 % CI, 1.8–5.8) were independent risk factors for death. The authors concluded,

Table 2.1 Biomarkers in penile cancer

Biomarkers	Number of studies	Function	Prognosis
p53	6	Tumor suppressor gene	Expression indicated higher risk of LN metastasis, disease progression, and worse DSS
$p16^{INK4a}$	5	Surrogate marker for high-risk HPV infection	Positivity was associated with less tumor invasion, lower risk of disease recurrence, and possibly better survival
Ki-67	4	Marker for tumor cell proliferation in the cell cycle	Labeling correlated with higher tumor grade, advanced local tumor stage, a greater risk of nodal metastasis, and clinical disease progression
PCNA	2	Marker of cell proliferation essential for replication	Expression was associated with presence of nodal metastasis
CRP	3	Pro-inflammatory marker	Elevated plasma levels found more often in patients with advanced tumor stage, positive nodal disease, and worse DSS
Cyclin D1	2	Regulates progression of cells through G1-phase of the cell cycle	No clear prognostic value; implicated in tumor differentiation
E-cadherin	1	Maintains cellular adhesion and signal transduction	Immunoreactivity was associated with a greater risk of LN metastasis
MMP-2 and MMP-9	1	Degrades the basement membrane of a cell	Immunoreactivity was associated with a greater risk of disease recurrence
Fox-P3	1	Oversees the development and function of regulatory T cells	Increased levels correlated to a lower inflammatory infiltrate worse OS
ARID1A	1	Involved in chromatin remodeling	Higher expression was associated with a higher histologic grade

therefore, that immunoreactivity of p53 was an independent risk factor for LN metastasis, and the association of positive p53 with positive HPV DNA was related to a worse prognosis.

Martins et al. [40] reported that p53 staining exhibited correlation with penile tumor pT stage ($P=0.0005$), grade ($P=0.02$), lymphatic spread ($P=0.02$), and CSS ($P=0.003$) in 50 patients with penile SCC [40]. Multivariate analysis showed that p53 immunoreactivity was the only risk factor with prognostic significance for disease progression and CSS. Since p53 overexpression was associated with tumor

progression and CSS, the authors argued that it should be evaluated in staging and therapeutic planning for patients with SCC of the penis.

Gunia et al. [41] showed p53 was an independently significant prognostic factor for CSS in penile cancer patients (hazard ratio [HR], 3.20; $P=0.041$) indicating worse prognosis. Zargar-Shoshtari et al. [42] reported that positive p53 status on immunohistochemistry was associated with pN+ disease (odds ratio [OR], 4.4; 95 % CI, 1.04–18.6) [42]. Liu et al. [43] also studied risk factors for the presence of pelvic LN metastasis in penile SCC patients undergoing inguinal lymph node dissection (ILND). Primary tumor strong p53 expression was a significant predictor of pelvic LN metastasis and OS (OR, 5.997; 95 % CI, 1.62–22.3). Finally, Zhu et al. [44] reported that the expression of p53 was an independent predictor of CSS in Chinese patients with penile cancer, and in stage T1 tumors, high expression of p53 was significantly associated with metastasis and poor survival.

$p16^{INK4a}$

Up to 50 % of penile SCC develops in the context of high-risk HPV infection [45]. Most of these tumors have been reported to show basaloid differentiation, and overexpression of the tumor suppressor protein $p16^{INK4a}$ is seen [46]. Whether HPV-triggered carcinogenesis in penile SCC has an impact on tumor aggressiveness, however, is still subject to debate with $p16^{INK4a}$ overexpression often used as a surrogate marker for high-risk HPV infection [8].

Steinestel et al. [47] analyzed tissue specimens from 58 patients with surgically treated penile SCC and performed $p16^{INK4a}$ immunohistochemistry and DNA extraction followed by HPV subtyping using a PCR-based approach. The sensitivity and specificity of $p16^{INK4a}$ staining to predict the presence of high-risk HPV DNA were 100 % and 57 %, respectively, and by focusing on samples with intense nuclear staining patterns for $p16^{INK4a}$, specificity could be improved to 83 %. Both expression of $p16^{INK4a}$ and presence of high-risk HPV DNA, but not histologic grade, were inversely associated with penile SCC tumor invasion ($P=0.01$, $P=0.03$, and $P=0.71$). However, none of these correlated with nodal involvement or distant metastasis. In contrast to pathological tumor stage, the high-risk HPV status, histologic grade, and $p16^{INK4a}$ positivity failed to predict CSS. These results confirmed that intense nuclear positivity for $p16^{INK4a}$, rather than histologic subtype, was a good predictor for the presence of high-risk HPV DNA in penile tumors. High-risk HPV/$p16^{INK4a}$ positivity, independent of histological tumor grade, indicated a less aggressive local behavior, but its value as an independent prognostic indicator remains to be determined.

Bezerra et al. [48] also showed a significant association of $p16^{INK4a}$ overexpression and high-risk HPV status with histologic subtype ($P=0.017$ and $P=0.01$, respectively) and lymphovascular invasion (LVI) ($P=0.015$ and $P=0.015$, respectively). Regarding survival outcome analyses, neither HPV infection nor $p16^{INK4a}$ overexpression significantly predicted OS or CSS using Cox proportional hazards regression model.

Ferrándiz-Pulido et al. also showed that strong $p16^{INK4a}$ immunostaining correlated with high-risk HPV infection [49]. Both high-risk HPV-positive and $p16^{INK4a}$-positive tumors showed a better OS without reaching statistical significance. The authors argued that routine use of $p16^{INK4a}$ staining should be incorporated in histologic evaluation of penile SCC.

Tang et al. [50] evaluated $p16^{INK4a}$ overexpression by immunohistochemistry for 119 consecutive patients with penile SCC[50]. $P16^{INK4a}$ overexpression was detected in 49.5 % (59 of 119) of samples. There was no significant difference between $p16^{INK4a}$ negative and $p16^{INK4a}$ positive tumors in terms of stage ($P=0.518$), histological grade ($P=0.225$), LVI ($P=0.388$), OS ($P=0.156$) or LN metastasis ($P=0.748$). $P16^{INK4a}$ negative tumors were more likely to recur overall ($P=0.04$), especially if patients had positive LNs at diagnosis ($P=0.002$). These data suggest that $p16^{INK4a}$/high-risk HPV status is associated with recurrence, especially in patients with positive LNs at diagnosis. Thus, patients with $p16^{INK4a}$ negative penile cancer, particularly those with LN metastases, may warrant closer observation after surgery.

Finally, Zargar-Shoshtari et al. [42] reported that in 57 cases of invasive penile SCC, estimated OS was insignificantly longer in $p16^{INK4a}$-positive patients (median OS, 75 vs. 27 months; $P=0.27$) and median CSS was not reached ($P=0.16$). In a multivariable Cox proportional hazard model, when controlling for pathological nodal status and adjuvant chemotherapy, $p16^{INK4a}$ status was a significant predictor for improved CSS (HR, 0.36 [95 % CI, 0.13–0.99]). Only one study has shown alterations in the tumor suppressor gene $p16^{INK4a}$ that are associated with aggressive behavior of penile carcinomas [51].

Ki-67

Ki-67 is a nuclear matrix protein expressed in the cell cycle phases that is a marker for tumor cell proliferation [52]. Its expression can be detected by immunohistochemistry. Its prognostic value in penile carcinoma is still considered controversial.

In a retrospective study of 44 patients in whom primary SCC of the penis was treated with amputation and bilateral lymphadenectomy (pT1 in 24, pT2 in 20, pN+ in 10; G1 in 12, G2 in 28, and G3 in 4), there was a tendency for high Ki-67 expression to be associated with advanced local tumor stage, nodal metastasis, and clinical disease progression, but these correlations were not statistically significant ($P=0.07$, 0.07, and 0.06, respectively) [53]. The authors concluded, therefore, that Ki-67 labeling may correlate with tumor grade in penile cancer and indicate a greater risk of nodal involvement.

The prognostic significant of Ki-67 was further supported by a small study from 73 Chinese patients who had penile amputation and regional lymphadenectomy [44]. LN metastasis was significantly correlated with tumor stage, histological grade, presence of LVI, and the expression of Ki-67. On multivariate analysis, presence of LVI and the expression of p53 were independent predictors of metastasis. Survival analysis showed that the expression of p53 was an independent prognostic

factor for CSS. In stage T1 tumors, high expression of p53 was also significantly associated with metastasis and poor survival.

Another study found a similar association between Ki-67 and tumor grade with Ki-67 expression notably increased with advanced tumor grade ($P<0.01$), but no association was found with tumor stage ($P=0.22$), presence of nodal metastasis ($P=0.74$), CSS (HR, 1.00; 95 %, CI, 0.99–1.02; $P=0.54$), or OS (HR, 1.00 95 %, CI, 0.99–1.02; $P=0.45$) [54]. High tumor stage, LN status, high tumor grade, and age at diagnosis were all independent prognostic factors for CSS and OS in this study.

May et al. [55] evaluated 158 consecutive patients with surgically treated penile SCC. Ki-67 displayed a significant positive correlation with histological tumor grade, LVI, and nodal status. On multivariable analysis, however, only pathologic tumor stage (HR, 1.67; $P=0.003$) and nodal stage (HR, 2.62; $P=0.015$) as well as tumor grade (HR, 1.89; $P=0.036$) and LVI (HR, 2.66; $P=0.028$) were identified as independent prognostic parameters for CSS. The authors concluded, therefore, that Ki-67 adds little to conventional histopathological criteria as powerful predictors of CSS in surgically treated penile carcinoma. It may just represent a marker of more aggressive behavior in this disease.

Finally, Guimaraes et al. [56] found an inverse relationship between Ki-67 and LN metastasis with low expression correlating with LN involvement. Ki-67 immunohistochemical expression also did not have an association with survival and death risk.

Proliferating Cell Nuclear Antigen

Proliferating cell nuclear antigen (PCNA) is another marker of cell proliferation found in the nucleus that is essential for cell replication. Martins et al. [40] found that PCNA expression was significantly associated with nodal disease ($P=0.04$) on univariate analysis, but it had no prognostic significance for nodal metastases, disease progression, or cause-specific death on multivariate analysis.

Guimaraes et al. [56] retrospectively evaluated 125 patients with penile SCC and found PCNA was an independent prognostic factor for LN metastasis but not CSS. Since there is no standardization in the execution and interpretation of PCNA, making comparison of results is challenging.

C-Reactive Protein

C-reactive protein (CRP) is produced by the liver in response to an inflammatory stimulus involving increased cytokine expression. It is elevated during malignancy by various mechanisms including inflammation caused by tumor growth, immune response, to tumor antigen, or the chronic inflammation itself, which can be the source for carcinogenesis. High plasma CRP has been linked to poor prognosis in other genitourinary malignancies including renal cell carcinoma and urothelial carcinoma, and dynamic changes in CRP concentrations over time could predict tumor aggressiveness and potential treatment efficacy [57].

Several studies have evaluated serum CRP levels as a prognostic marker in penile cancer. Steffens et al. [58] retrospectively analyzed 79 patients with information about their serum CRP value prior to surgery who underwent either radical or partial penectomy. A significantly elevated CRP blood level (>15 vs. ≤15 mg/L) was found more often in patients with an advanced tumor stage (≥pT2) (38.9 % vs. 11.6 %; $P=0.007$) and in those with nodal disease at diagnosis (50.0 vs. 14.6 %; $P=0.007$). High CRP levels, however, were not associated with tumor grade ($P=0.53$). The 5-year CSS rate was 38.9 % for patients with preoperative CRP levels above 15 mg/L and 84.3 % for those with lower CRP levels ($P=0.001$). Applying multivariate analysis and focusing on the subgroup of patients without metastasis at the time of penile surgery, both advanced local tumor stage (≥pT2; HR 8.8; $P=0.041$) and an elevated CRP value (>15 mg/L; HR 3.3, $P=0.043$) were identified as independent predictors of poor clinical outcomes in patients with penile cancer. A high preoperative serum CRP level, therefore, was associated with poor survival in patients with penile cancer.

Al Ghazal et al. [59] studied 51 penile cancer patients and found that high presurgical CRP levels were significantly associated with the diagnosis of nodal involvement ($P=0.04$) [59]. The optimal CRP cut-off value to predict LN metastasis was set at 20 mg/L based on ROC analysis. Since a high preoperative serum CRP level was closely correlated with nodal disease, it could be used as an additional marker to help identify patients with penile cancer who may benefit from ILND.

Finally, Li et al. [60] evaluated the association between pretreatment levels of CRP and SCC antigen (SCC-Ag) on 124 Chinese penile SCC patients treated between November 2007 and October 2014. Levels of CRP ≥4.5 mg/L and SCC-Ag ≥1.4 ng/mL were both significantly associated with LN metastasis laterality ($P=0.041$), extranodal extension (ENE) ($P<0.001$), pelvic LN metastases ($P=0.024$), pathologic tumor status ($P=0.002$), pathologic nodal status ($P<0.001$), and disease-specific survival (DSS) ($P<0.001$). Moreover, the influence of CRP and SCC-Ag levels on CSS ($P=0.033$; HR, 3.390; 95 % CI 1.104–10.411) remained after adjusting for smoking history, phimosis, tumor status, tumor cell differentiation, and nodal status. The combined measurement of preoperative CRP and SCC-Ag levels may serve as an independent biomarker for LN metastasis, advanced tumor stage, and DSS in penile SCC patients.

Cyclin D1

Cyclin D1 plays an important role as a cell cycle activator in regulating the progression of cells through the G1-phase of the cell cycle. Cyclin D1 overexpression may be used as a prognostic factor of poor outcome in penile carcinoma.

Papadopoulos et al. [61] evaluated 21 penile SCC patients and a tendency for an association between cyclin D1 expression and tumor differentiation ($P=0.07$), but not the level of tumor invasion ($P=0.50$) was found. Gunia et al. also analyzed the role of p53, p21, and cyclin D1 expression in patients with penile cancer [41]. Specimens and clinical data from 110 men treated surgically for primary penile

cancer were collected. Multivariable analysis showed p53 (HR, 3.20; $P=0.041$) and pT-stage (HR, 4.29; $P<0.001$) as independent significant prognostic factors for CSS. Cyclin D1 and p21 expression were not correlated with survival. However, incorporating p21 into a multivariable Cox model did contribute to improved model quality for predicting CSS.

Other Biomarkers

Other biomarkers in penile cancer have been sparsely studied. In a study by Campos et al. [62], 125 penile cancer patients who had undergone primary penile tumor excision and bilateral lymphadenectomy had their tissues stained for the presence of E-cadherin, matrix metalloproteinase (MMP)-2, and MMP-9 [62]. E-cadherin is a protein responsible for maintaining cellular adhesion and signal transduction, while MMPs help degrade the basement membrane of a cell resulting in tumor metastasis. The authors reported that low E-cadherin immunoreactivity was associated with a greater risk of LN metastases on univariate analysis ($P=0.03$), and high levels of MMP-9 immunoreactivity were independently associated with a greater risk for disease recurrence on multivariate analysis ($P=0.02$).

Since there is growing evidence that immune cells may trigger various mechanisms that enhance tumor growth and metastasis, this same group also evaluated the immunohistomorphology of peritumoral inflammation in penile cancer and correlated it with clinical and pathological parameters [63]. Fox-P3 is a master transcription factor protein that oversees the development and function of regulatory T cells, which generally turns the immune response down. As such, an increase in Fox-P3 expression can result in excess regulatory T-cell activity and prevents the immune system from destroying cancer cells. In the study mentioned above, Vassallo et al. [63] correlated increased levels of Fox-P3-positive lymphocytes with a lower inflammatory infiltrate and subsequently more unfavorable 5-year OS in penile cancer patients.

Finally, ARID1A, a member of the chromatin remodeling genes family, has been suggested as a novel tumor suppressor gene in gynecologic malignancies, but its role in penile cancer is largely unknown. Faraj et al. assessed the immunohistochemical staining of ARID1A in 112 cases of penile SCC from Paraguay and found ARID1A expression in 90% or more of tumor cells in over 85% of cases [64]. There was also a significant correlation between higher ARID1A expression and higher histologic grade, but there was no association with HPV status or the risk of LN metastasis.

Conclusions

Novel diagnostic tools in the evaluation and management of penile cancer continue to be developed to enhance patient selection for both PSS and lymphadenectomy in order to minimize treatment-related morbidity and improve overall QOL.

Future studies will further enhance these imaging-based modalities or tissue-based biomarkers to provide further information of a patient's clinical status and create a more patient-centered approach to treatment.

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