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Penile cancer: prognostic and predictive factors in clinical decision-making

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Abstract. – Penile cancer (PC) is a typical tumor of non-industrialized countries. The incidence is 20-30 times higher in Africa and South America, considering the elevated prevalence of sexually transmitted diseases. Histologically, PC includes squamous cell carcinoma (SCPC), the most frequent, and nonsquamous carcinoma (NSCPC). Early diagnosis is the goal, whereas later diagnosis relates to poor functional outcomes and worse prognosis. The 5-year survival rate is 85% for patients with histologically regional negative lymph nodes, compared to 29%-40% for those with histologically regional positive lymph nodes. To date no new drugs are approved, and there are few new data about molecular mechanisms underlying tumorigenesis. The SCPC remains a rare tumor and the current therapeutic algorithm is based principally on retrospective analysis and less on prospective trials. In this review article, biomarkers of prognosis and efficacy of current treatments are summarized with a focus on those that have the potential to affect treatment decision-making in SCPC.

Key Words:
Penile, Cancer, Prognosis, Metastasis, Treatment.

Introduction

Penile cancer (PC) is a typical tumor of non-industrialized countries. The incidence is 20-30 times higher in Africa and South America, considering the higher prevalence of sexually transmitted diseases[1,2]. Histologically PC includes squamous cell carcinoma (SCPC), the most frequent, and nonsquamous penile carcinoma (NSCPC). SCPC occurs predominantly in elderly men with a median age of 60 years old and represents the 0.4-0.6% of all cancer in USA and Europe[3-7]. Early diagnosis is the the goal, because later diagnosis result in poor functional outcomes and worse prognosis. The 5-year survival rate is 85% for patients with histologically regional negative lymph nodes, compared to 29%-40% for those with histologically regional positive lymph nodes. The pelvic lymph node involvement is associated with the lowest survival rates. Surgery remains the milestone in localized tumor, conversely chemotherapy represents the standard of care in advanced cancer. To date no new drugs are approved, and there are few new data about molecular mechanisms underlying tumorigenesis[7]. The SCPC remains a rare tumor and the current therapeutic algorithm is based principally on retrospective analysis and less on prospective trials. In this review article, biomarkers of prognosis and efficacy of current treatments are summarized with a focus on those that have the potential to affect treatment decision-making in SCPC.

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Histology and Clinical Prognostic and Predictive Factors

The SCPC is the histologically predominant variant (95%). Other histologic types include melanomas, basal cell carcinomas, sarcomas, adenosquamous, mixed forms, and poorly differentiated types, extremely rare (5%)[7-13]. The prognostic role of the histology was confirmed by several studies that showed as the basaloid, sarcomatoid and adenosquamous variants correlated with poorly differentiated types and deep tissue infiltration, conversely the verrucous, papillary and condylomatous (warty) variants were associated with low grade tumor and superficial invasion. The 10-year survival rate are 100%, 100%, 97%, 92%, and 90%, for the verrucous, adenosquamous, mixed, papillary and warty carcinoma, respectively, while patients with the squamous and basaloid types have 78% and 76% 10-year survival, respectively. Unfortunately, 75% of patients with sarcomatoid variants died within one year of diagnosis[14-18]. As expected, poorly differentiated tumor and lympho-vascular invasion on tumor sample correlate positively with local or systemic recurrence[19].

PC is a highly curable disease when diagnosed on early stage (0, I, and II stage), instead advanced disease (III and IV stage) remains hardly to cure. The estimated 10-year survival is 89% for stage I, but only 21% of stage IV are alive at 2 years from diagnosis[4]. Pathologic TNM staging remains the main prognostic factor after surgery (Tables I, II III, IV)[20-22]. Regional lymph nodes (LNs) involvement correlate with overall survival (OS). The reported 5-year survival ranged from 80-90% for patients with unilateral inguinal LNs involvement, to 10-20% in case of bilateral inguinal LNs metastases or pelvic LNs involvement[4,5,23-26]. Leijte et al[27] showed a higher (27.7%) incidence of local recurrence after penile-preserving surgery than amputation (5.3%), although this difference did not translate into longer OS, because of salvage surgery. The regional recurrence rate was 2.3% in pN0 *vs.* 19.1% in pN+. The 5-year disease specific survival rate was 92% after a local recurrence and 32.7% after regional recurrence, while all patients with a systemic recurrence died within 22 months. Most of tumor recurrences (86%) occurred early, within 2 years[27]. The regional LNs metastases negatively affect the OS. At diagnosis, clinically palpable LNs are present in 28%-64% of patients, of which 47%-85% are histologically confirmed metastases, and 12-20% are due to inflammatory reactions; conversely, 12%-20% of patients without clinical palpable inguinal LNs have histologically confirmed metastases, after surgery[5]. Visceral metastases occur later, usually in patients with histologically inguinal and pelvic LNs metastases. The presence of extracapsular tumor invasion in inguinal or pelvic LNs appear to be independently associated with decreased 5-year cancer-specific survival (42% and 22%, respectively)[28,29]. Several nomograms were performed to better predict cancer-specific survival and LNs metastases. A retrospectively analysis of the clinical and pathological data of 175 resected SCPC patients, showed that the presence of palpable inguinal LNs and the presence of histologically confirmed vascular and/or lymphatic tumoral invasion, predicted LNs tumoral involvement[30]. A prospectively study of 106 patients with SCPC showed that high tumoral grade (p=0.004), lympho-vascular tumoral invasion (LVI) (p=0.01) and clinical palpable inguinal LNs (p=0.05) correlated positively with tumoral metastases[31]. The following factors were identified as independent predictors of pathologic LNs metastases: 1) clinical LNs status, 2) pathologic TNM stage of the primary tumor, lympho-vascular tumoral invasion, and tumoral differentiation grade[32]. Kattan et al[33] elaborated two nomograms to predict SCPC specific OS. The first model was based on the pathological characteristics of primary tumor after penectomy and on the clinical stage of inguinal LNs, while the second model included the pathological data of both the primary tumor and inguinal LNs. The concordance index was 0.728 for the first model and 0.747 for the second one[33]. Other studies[34-37] have identified LNs density, and/or the lack of koilocytosis and/or the clear cell subtype as important prognostic factors. The lymph node ratio (LNr) is defined as the ratio of the histologically positive LNs metastases to the total number of removed LNs. The role of the LNr as prognostic factors, was extensively explored in bladder cancer[38,39]. Interestingly, Lughezzani et al[40] evaluated the correlation between the LNr and the cancer-specific survival (CSS). The 5-year CSS rates was 65.2% *vs.* 9.6% in patients with LNr < 22% and ≥ 22%, respectively (p<0.001). In a multivariable Cox regression models, the LNr was an independent predictor of CSS (p≤0.012). Burt et al[41] evaluated the CSS and demonstrated that G2-3 disease, T3 stage, and positive LNs were adverse prognostic factors for CSS41. Recetly, Li et al[42] indicated the significant prognostic value of lympho-vascular embolization for metastasis and surviv-

Table I. Primary tumour (T).

TX	***Primary tumor cannot be assessed.***
T0	No evidence of primary tumor.
Tis	Carcinoma in situ.
Ta	Noninvasive verrucous carcinoma
T1a	Tumor invades subepithelial connective tissue without lymph vascular invasion and is not poorly differentiated (i.e., grade 3-4).
T1b	Tumor invades subepithelial connective tissue with lymph vascular invasion or is poorly differentiated.
T2	Tumor invades corpus spongiosum or cavernosum.
T3	Tumor invades urethra.
T4	Tumor invades other adjacent structures.
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	Palpable fixed inguinal nodal mass or pelvic lymphadenopathy unilateral or bilateral.
Clinical stage definition	
pNX	Regional lymph nodes cannot be assessed.
pN0	No regional lymph node metastasis.
pN1	Metastasis in a single inguinal lymph node.
pN2	Metastases in multiple or bilateral inguinal lymph nodes.
pN3	Extranodal extension of lymph node metastasis or pelvic lymph node(s) unilateral or bilateral.
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	Palpable 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 a single inguinal lymph node.
pN2	Metastases in multiple or bilateral inguinal lymph nodes.
pN3	Extranodal extension of lymph node metastasis or pelvic lymph node(s) unilateral or bilateral.

Table II. 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	Palpable 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 a single inguinal lymph node.
pN2	Metastases in multiple or bilateral inguinal lymph nodes.
pN3	Extranodal extension of lymph node metastasis or pelvic lymph node(s) unilateral or bilateral.

Table III. Distant metastasis (M).

M0	No distant metastasis.
M1	Distant metastasis.

al (all $p<0.001$); furthermore, they porposed a modified clinicopathological staging system with the T2 and T3 categories of the 8th AJCC-TNM staging system being subdivided into two new categories as follows: T2 tumors invade the corpus spongiosum and/or corpora cavernosa and/or urethra without lymphovascular invasion, and T3 tumors invade the corpus spongiosum and/or corpora cavernosa and/or urethra with lympho-vascular invasion. The modified staging system involving lympho-vascular embolization showed improved prognostic stratification with significant differences in CSS among all categories (all $p<0.005$) and exhibited higher accuracy in predicting patient prognoses than did the 8th AJCC-TNM staging system (C-index, 0.739 *vs.* 0.696). Squamous cell carcinoma antigen (SCC-Ag) is a well-known marker for various carcinomas. The analysis of SCC antigen in 54 SCPC patients at different disease stages seemed to correlate with tumor burden, increasing significantly only after massive lymph node involvement or metastatic disease[43]. In this regard, Li et al[44] showed that preoperative levels of C-reactive protein (CRP) $\geq$ 4.5 mg/L and SCC-Ag $\geq$ 1.4 ng/mL were both significantly associated with LNs metastases ($p=0.041$), extra nodal extension ($p< 0.001$), pelvic LNs ($p = 0.024$), pathological tumor status ($p=0.002$), pathological nodal status ($p<0.001$), and disease-specific survival (DSS; $p < 0.001$). Moreover, the influence of CRP and SCC-Ag levels on DSS ($p= 0.033$) remained after adjusting for smoking history, phimosis, tumor status, tumor cell differentiation and nodal status.

Table IV. Anatomic stage/prognostic groups.

Stage	T	N	M
0	Tis	N0	M0
	Ta	N0	M0
I	T1a	N0	M0
II	T1b	N0	M0
	T2	N0	M0
	T3	N0	M0
IIIa	T1–3	N1	M0
IIIb	T1–3	N2	M0
IV	T4	Any N	M0
	Any T	N3	M0
	Any T	Any N	M1

Etiology and Biological and Molecular Prognostic and Predictive Markers

The main risk factors associated with PC are as follows: balanitis, chronic inflammation, penile trauma, tobacco use, lichen sclerosus, poor hygiene and phimosis; among them, the phimosis correlated with increased risk for PC from 25% to 60%. Other risk factors include a history of sexually transmitted diseases, especially HIV and HPV infection[45-61], although the latter remains the best known. Several scholars[54] have identified high-risk (HR-HPV) and low-risk HPV (LR-HPV) strains, classified by their oncogenicity. High-risk strains include the 16, 18, 33, and 35, while low-risk include the 6 and 11. HR-HPV infect nonkeratinized squamous mucosa, like cervix, anus, and oropharynx, but not keratinizing squamous epithelium of the skin. The prevalence of HPV infection in adult men appears to be constant across age groups, without difference between younger and older men[55]. HPV infection can result in a spectrum of genitourinary manifestations, including genital warts, penile intraepithelial neoplasia (PIN), up to PC. PIN represents a dysplastic pre-malignant lesion, and it is subdivided into: erythroplasia of Queyrat (EQ), Bowen disease (BD), and bowenoid papulosis. A systematic review[61] evaluating HPV prevalence in PC, found that 48% of analyzed tumors were positive for HPV. The most common HPV strains identified were the 16 and 18. HPV prevalence varied significantly among PC histologic subtypes[61]. It has been showed that only 22.4% of verrucous SCC result positive for HPV compared to 66.3% of basaloid/warty subtypes. These data were recently confirmed by D'Hauwers et al[62] who showed that overall HPV DNA was found in 70.9% of 76 samples of penile lesions, of which 89.5% in PIN (n=19) and 61.1% in PC (n=36). Poorly differentiated, basaloid, warty-basaloid, and warty carcinomas are more consistently associated with HPV infection, suggesting that distinct pathogenic pathways may drive tumorigenesis[58,59,63]. The pathogenetic mechanisms of HPV tumorogenesis are unclear. The inactivation of the tumor suppressors p53 by HPV-E6 and Rb by HPV-E7 play a key role in HPV oncogene associated carcinogenesis, affecting negatively the cell cycle regulation[64]. Particularly, E7 activity on tumor suppressor Rb, blocks the feedback inhibition on p16Ink4a, resulting in increased expression of p16Ink4[65]. In many studies was evaluated p16 (INK) immunohistochemical expression, as a potential marker

of HR-HPV infection. p16 (INK) overexpression is a marker for HR-HPV infection and many data confirmed this result[63]. HPV infection prevalence correlated with clinical outcome. Djajadiningrat et al[66] showed that the 5-year DFS in the HR-HPV negative group and in the HR-HPV positive group was 82% and 96%, respectively (log rank test p=0.016); after adjusting for pathological stage, tumoral grade, lympho-vascular invasion and age, HPV status was confirmed as prognostic factors (p=0.030) with a HR of 0.2 (95% CI 0.1-0.9)[66]. To confirm this, McDaniel et al[67] evaluated 60 fixed tumor samples from 43 SCPC, and they found a p16 overexpression in 28% of patients, including all HPV-positive cases; of note, p16 positivity was significantly associated with longer event-free survival (combined progression or PeSCCA-specific death). Previous studies[66,68] have generally shown that HPV status and p16 positivity correlated with favourable prognosis. Conversely, Lopes et al[69] revealed that only lymphatic tumoral invasion (RR: 9.4) and p53 positivity (RR: 4.8) were independent factors for lymph node metastases; patients with negative p53 had significantly better 5 and 10-year OS *vs.* positive p53 tumors (64.5% and 54.6% *vs.* 30.2% and 26.4%, respectively, p = 0.009). In addition, the p53 positive tumors combined with HPV DNA positive, correlated with the the poorest OS[69]. The genomic landscape of SCPC is only partially understood, with a limited number of aberrant detected genes, primarily p53, CDKN2A, EGFR and inhibitor of DNA binding 1 (ID1)[68,70,71]. A comprehensive genomic profiling (CGP) was performed to identify clinically relevant genomic alterations (CRGAs). This analysis revealed 109 genomic alterations (Gas) (5.45 per tumor), 44 of which were CRGAs (2.2 per tumor). At least one CRGA was detected in 19 (95%) cases, and the most common CRGAs were CDKN2A point mutations and homozygous deletion (40%), NOTCH1 point mutations and rearrangements (25%), PIK3CA point mutations and amplification (25%), EGFR amplification (20%), CCND1 amplification (20%), BRCA2 insertions/deletions (10%), RICTOR amplifications (10%), and FBXW7 point mutations (10%). Less frequent alterations in these series included FGF amplification and mutation of chromatin remodeling genes[72]. Poetsch et al[73] studied 62 microsatellite repeats from 11 different chromosomes in 28 SCPC and 10 corresponding metastases for allelic imbalances and loss of heterozygosity (LOH) looking for molecular genetic characteristics important for progression and clinical outcome. LOH was found in more than 25% of primary tumors on six different chromosomes, including 2q, 6p, 8q, 9p, 12q and 17p13, suggesting the presence of important tumor suppressor genes in these regions[73]. LOH in the chromosomal loci 6p22-23 was significantly associated with a poor prognosis among SCPC patients. Tumors with LOH in the region of p16INK4a (localized in the 9p21 region), showed a significant higher risk for LNs metastases (p=0.005)[73]. Interestingly, the basaloid variants showed a relatively small number of LOH compared with poorly differentiated sarcomatoid carcinoma. Aleves et al[74] performed a comparative genomic hybridization study of 26 cases of SCPC. DNA sequence copy number alterations (CNAs) resulted similar to those detected in other SCC types, such as oral and esophageal tumors. The most common copy number gains were found in the 8q24, 16p11-12, 20q11-13, 22q, 19q13, and 5p15 chromosome, and the most common deletions were detected in the 13q21-22, 4q21-32, and along the X chromosome. By classifying patients according to the number of CNAs, they showed a possible correlation with clinical outcome[74]. Interesting gains in copy number were frequently reported within the 8q24 chromosomal region. The proto-oncogene MYC was located in this region and several studies have demonstrated that the insertion of HPV16 DNA within this region, resulting to an over amplification of the MYC[75]. MYC overexpression and CCND1 amplifications were associated with poor cancer-specific outcome, with decreased event-free survival[67,74,75]. TP53, CDKN2A, PIK-3CA, MYC, HRAS, and SOX2 were the most frequently altered genes. No significant correlations were present between mutation status for a specific gene and tumor grade, stage, or histology[67]. The mutational burden was significantly less in HPV positive *vs.* HPV negative SCPC, and no HPV-positive SCPC harboured neither TP53 alterations nor EGFR amplifications, unlike SCCs of other sites[67]. Different studies[76-78] evaluated the overexpression of the EGFR as a potential biomarker and target of biological therapy. SCPC primary tumours and metastases highly express EGFR, with a frequency of 91%-100%. The members of this family are EGFR, HER2, HER3, and HER4 transmembrane tyrosine kinase receptors, and their activation cause phosphorylation of tyrosine residues (p-EGFR) with a subsequent activation of a several downstream pathways, including the PI3K/Akt and

Ras-Raf-MEK-ERK. Di Lorenzo et al[79] evaluated 30 PC tissue samples. All specimes were positive for EGFR by immunohistochemistry, while only 13 and 16 were positive for nuclear and cytosolic p-EGFR, respectively. FISH detected no EGFR amplification. Expression of p-EGFR strongly correlated with an increased recurrence risk and a shorter OS. HPV-negative tumors tended to express significantly more pEGFR than HPV-positive cancers and this expression correlated with pAkt protein, indicating EGFR as an upstream regulator of Akt signaling in SCPC. Conversely, HER3 expression was significantly more common in HPV-positive tumors and positively correlated with cytoplasmic Akt1 expression. HER4 and PTEN protein expression were not related to HPV infection[80]. Silva Amancio et al[81] confirmed the negative association between EGFR overexpression and cancer recurrence (p=0.004) and perineural invasion (p=0.005). Interestingly, the same authors failed to identify any of the activating mutations in the tyrosine kinase domain of EGFR known to be implicated in lung cancer, such as EGFR E746 - A750-specific deletion in exon 19 and EGFR L858R specific point mutation in exon 21. The absence of known mutations on EGFR, as in lung cancer, and on RAS, like colon-rectal cancer, was confirmed by Gou et al[82] who found KRAS mutation in only one (1/94) sample and found no BRAF V600E point mutation.

See comment in PubMed Commons belowThe proliferation marker Ki67 has been shown to be highly expressed in more aggressive SCPC and its expression was associated with poorer survival[83]. De Paula et al[84] evaluated the histological and cyclooxygenase-2/vascular endothelium growth factor-C (COX-2/VEGF-C) immunohistochemical profiles of 127 PC and showed that VECF-C expression was associated with unfavorable clinical outcome, but not COX-2 expression. Inguinal LNs metastases and advanced stage were independent prognostic factors for poorest OS[84]. The main limit of this molecular analysis and its use in clinical practice was the significant intratumor and inter-tumor (metastases) heterogeneity. Several analyses suggest that multiple and complex interactions occur between the primary tumor and metastatic sites, and the coexistence of various sub-clones with different prognosis, particularly in advanced stage. Estimation of driver gene prevalence based on single regional sequencing significantly under-estimates the true molecular tumor-assessment.

Treatment

Loco-Regional Involment and Management Surgery

SCPC was staged according to the American Joint Committee on Cancer TNM Cancer Staging Manual[4]. The surveillance is the best treatment in patients diagnosed in early stage (Tis, Ta and T1), with favorable prognostic factors (i.e., Grades 1 or 2) without palpable LNs. Laser ablation is particularly indicated for small glans tumors in which margins ≥3 mm can be attained. The standard of care for non-invasive SCPC remains the use of topical medications, such as 5-fluorouracil (5-FU) or imiquimod, laser ablation or local excision. Penile sparing surgery or glans-sparing procedures (limited excision with or without circumcision, Mohs micrographic surgery, laser ablation and radiotherapy) are appropriate and safe options for Tis and T1 SCPC, also, in case of recurrent Tis, allowing penile function preservation with a lower psychosocial impact and excellent oncological outcome. For large tumors (≥T2) a total penectomy remains the gold standard, although in some T2 tumors, based on localization, partial penectomy is amenable[7,85,86]. An accurate staging of the primary lesion is essential to plan the best treatment protocol and to prognosticate the risk of associated LNs metastases.

– Patients without palpable LNs, a risk-stratified approach can be used to decide the better management of the inguinal region. In clinically node-negative patients (cN0), LNs micrometastases occur in about 25% of cases and correlate with tumor stage and grade. Early inguinal lymphadenectomy offers higher long-term patient survival compared to salvage lymphadenectomy in case of regional recurrence[87,88]. These data were confirmed by a prospective trial, reporting a five-year OS significantly better with inguinal lymphadenectomy *vs.* immediate inguinal radiotherapy or surveillance strategy (74% *vs.* 66% and 63%, respectively)[89]. Very low-risk SCPC are pTa, pTis. pT1 tumors are a heterogeneous group, including low-risk tumors (pT1G1), intermediate-risk (pT1G2)[16], and high-risk (pT1G3, pT2-4 any G or any pTG3). Very low and low-risk patients could be observed, while bilateral superficial or complete modified inguinal nodal dissection should be the standard of care in the other risk groups. Recent studies[90-92] promoted the use of sentinel lymph node in cN0, with high sensitivity (90-94%) and lower morbidity.

– Patients with palpable LNs, fine-needle aspiration cytology (FNAC) is currently recommended. A core biopsy or excisional biopsy can also be performed. In case of inguinal LNs metastases, bilateral inguinal lymphadenectomy is indicated, with a significant morbidity[93]. Pelvic LNs should not be removed if inguinal LNs are negative. The pelvic LNs dissection is recommended in patients with multiple inguinal LNs metastases, or extra-nodal extension, or LNs of Cloquet involvement[94]. A total excision of the positive inguinal lymph nodes represents the main prognostic factor; therefore, it would be possible to remove the primary lesion and regional LNs at two different times. Otherwise, in case of palpable inguinal LNs greater than 4 cm, or fixed nodes, or radiological or clinical involvement of pelvic lymph nodes, a multimodal approach with systemic chemotherapy, surgery, and radiotherapy represent the standard of care. Unfortunately, many questions are open about the multimodal correct management of these clinical situations, and no published randomized trials are available. A recent retrospective analysis of the U.S. National Cancer Database (NCDB) showed that triple modality therapy (surgery (S) + chemotherapy (C) + radiotherapy (XRT) did not extend OS compared to dual modality therapy (S+C or S+XRT). Additionally, the analysis did not identify whether C or XRT should be preferred in pts receiving dual modality therapy[95]. The International Penile Advanced Cancer Trial (InPACT), is an ongoing study with the aim to determine prospectively the relative benefits and sequencing of surgery, chemotherapy, and chemoradiotherapy in the management of patients with penis cancer who present with palpable or radiologically evident inguinal LNs metastases[96].

Radiotherapy

Historically, SCPC has been considered radio-resistant tumors, considering the high dose (60Gy) required, with significant adverse events. However, in selected patients (T1-T2), an external radiation therapy (XRT) or brachytherapy could be an alternative to surgery, using a salvage resection in case of local recurrence. The role of adjuvant XRT is unclear. Burt et al[97] failed to demonstrate a significant positive effect in terms of CSS between surgery alone vs. surgery plus ERBT[97]. According to the EAU guidelines, adjuvant inguinal XRT may be considered as an option in selected high-risk patients[7,98]. Palliative radiation remains the standard in unresectable inguinal lymph node metastases[7]. The role of concurrent chemoradiotherapy for locally advanced SCPC is unclear. Recently, Pond et al[99] showed poor outcomes in this setting with the use of concurrent chemoradiotherapy, with a median OS and PFS of 10.0 months (95% CI, 5-14) and 6.0 months (95% CI, 2.0-7.0), respectively[99].

Adjuvant and Neoadjuvant Chemotherapy

The role of neoadjuvant or adjuvant chemotherapy in LNs metastases SCPC is unclear; there are only few, small and heterogeneous retrospective studies with inconclusive results and no randomized clinical trials was published. Fortunately, patients with three or fewer unilateral inguinal LNs metastases, without extranodal extension or pelvic LNs involvement, have a low rate of disease recurrence: 10% to 20% after surgery alone[100], conversely the recurrence rate is higher (80-90%) in patients with bilateral LNs metastases or extranodal extension, or pelvic LNs involvement[101,102]. Three Italian studies evaluated different combination chemotherapy, such as 12 weekly courses of vincristine, bleomycin and methotrexate (VBM), or 3 courses of cisplatin and 5-fluorouracil (5-FU) or 3-4 courses of taxane-based regimen (TPF), in clinical bulky and/or fixed LNs metastases SCPC (neoadjuvant chemotherapy) or after surgery (pN2-3) (adjuvant chemotherapy). The results were encouraging, suggesting that adjuvant chemotherapy could improve the long-term survival and neoadjuvant chemotherapy could make resectable approximately 50% of cases with fixed inguinal metastases[103-105]. The same results were confirmed by Noronha et al[106] who demonstrated that paclitaxel and platinum combination regimen was safe and effective, with an estimated median DFS of 16.2 months and a longer median OS. Nicolai et al[107] evaluated the efficacy of T-PF in the neoadjuvant and adjuvant setting, high-risk SCPC patients (cN2-N3 or pN2-3). The 2-year disease-free survivals (DFS) were 36.8% (95% CI, 15.2-58.5) *vs.* 7.1% (95% CI, 0-16.7) after adjuvant and neoadjuvant therapy, respectively. N3 metastases were associated with poorer DFS while, bilateral inguinal metastases or mutated p53 gene with a poorer OS. The neoadjuvant treatment, despite a 43% of clinical responses and a 14% of complete pathologic remissions, was not associated with longer OS.

These results were not confirmed by Djajadiningrat et al[108], who showed that, despite a good response percentage, TPF chemotherapy was poorly tolerated with disappointing survival rates[109]. Recently, Zargar-Shoshtari et al[110] evaluated the role of adjuvant chemotherapy (AC) in 141 SCPC patients who had positive pelvic LNs. At median follow-up of 12.1 months, the estimated median OS was 21.7 (IQR: 11.8-104) *vs*. 10.1 months (IQR: 5.6-48.1) in AC *vs*. no AC arm, respectively (p=0.048). AC was independently associated with improved OS on multivariate analysis (HR: 0.40; 95% CI: 0.19-0.87; p= 0.021). In patients with clinical multiple, fixed or bulky inguinal LNs (≥ 4 cm) or radiological/clinical pelvic LNs involvement, surgery alone achieved poor outcome. The neoadjuvant chemotherapy role is not completely elucidated. A multidisciplinary strategy should include primary chemotherapy followed by surgery with LNs resection if possible, eventually XRT also, as a consolidation treatment in high-risk resected SCPC (pN2-3). Different chemotherapy regimen, such as combination of bleomycin-vincristine-methotrexate (BVM) or bleomycin-methotrexate-cisplatin (BMP) or cisplatin/5-FU (PC), was evaluated without differences in terms of outcome, but with different toxicity profile, in favour of PC[111-114]. Pagliaro et al[115] evaluated the role of TIP as neoadjuvant treatment in cN2-3 SCPC patients; pCRs occurred in 13.6% of patients and resulted not statistically significant predictor of TTP (p=0.11), but marginally significant predictor of OS (p=0.07). Recently, Necchi et al[116] showed no significant differences in terms of OS (p= 0.45) between neoadjuvant *vs*. adjuvant *vs*. neoadjuvant and adjuvant chemetherapy. One-year relapse-free survival was 35.6%, 60.6%, and 45.1% in the 3 groups, respectively. One-year OS was 61.3%, 82.2%, and 75%, respectively. No significant differences were observed on univariable analyses for OS between the groups. Overall, the use of adjuvant combination chemotherapy regimen is recommended, for pN2-3 SCPC (LE:2b)[7]. No data for adjuvant chemotherapy in pN1 is available[7]. The use of neoadjuvant chemotherapy is recommended for clinical bulky or multiple or bilateral, or fixed inguinal LNs and/or unilateral or bilateral pelvic LNs metastases; particularly three-drug chemotherapy regimen, including cisplatin and taxane, should be the standard of care (LE: 2a)[7].

Chemotherapy for Advanced Disease

Treatment of metastatic SCPC is associated with poor outcomes with median OS of 6-12 months. Visceral metastases (VM) and ECOG PS ≥1 are valitaded as poor prognostic factors and correlated with shorter OS and PFS. Cisplatin-based regimen is associated with longer OS (p=0.017) but not PFS (p= 0.37), compared with non-cisplatin-based regimen[117]. The best first-line chemotherapy is unknown and different regimens are in use. Protzel et al[118] emphasized the non-uniformity of chemotherapy treatments in use, showing how eighteen different combination chemotherapy regimens were used in Germany, without a practice standardization. Combination chemotherapy with two or three drugs provide mixed results[7,85,119-123]. Several studies evaluated the association between cisplatin and 5FU. Di Lorenzo et al[124] showed a 32% of partial responses (PR) and 40% of stable disease (SD) with cisplatin and 5-FU continuous 24-infusion for 4 days in 25 SCPC patients. The median [interquartile range IQR)] PFS was 20 (11-20) weeks and the median (IQR) OS was 8 (7-12) months[124]. Recently, Theodore et al[125] evaluated the combination of cisplatin and irinotecan in 28 SCPC with 30.8% of RR. A phase II study evaluating the combination of gemcitabine and cisplatin without significant responses in patients with unresected locoregional or metastatic SCPC[125]. The combination of paclitaxel or docetaxel with cisplatin and 5FU showed the same results reached in the neoadjuvant setting[106-107]. There are not solid data in the second line chemotherapy. The presence of VM and Hb ≤ 10 gr/dl were associated with poor OS and PFS in second or later line chemotherapy[126]. Taxanes have been used with modest activity. In a prospective, multicenter phase II trial, 25 patients were enrolled and treated with paclitaxel 175 mg/m^2 every 3 weeks. Median PFS was 11 wk. (95% CI, 7-30); median OS was 23 wk. (95% CI, 13-48). Median survival in responders was 32 wk. (95% CI, 20-48)[127]. An ongoing phase II trial is evaluating the role of vinflunine in locally advanced and metastatic SCPC (VinCaP)[128]. Overall, Cisplatin-based chemotherapy with TIP or in combination with 5FU remains the standard of care. Carboplatin- based chemotherapy should not replace cisplatin; it could be an alternative in case of renal impairment or in elderly patients.

Novel Systemic Regimens and Biological Agents

Target Therapy

SCPC presents some molecular analogies with the other SCC, particularly with head and neck, esophageal, and cervix cancer. As we have already explained above many somatic gene alterations were found in SCPC samples. EGFR family, mTOR/Akt/PIK3CA, NOTCH1, CDKN2A, CCND1, AR, KAK2, JAK2, ALK, PTEN and BRCA2, represent potential targets for new drugs (targeted therapy-TT). Unfortunately, the small number of patients, the absence of multicenter collaboration or prospective clinical trials, limit the evaluation of these potential therapeutic targets. SCPC and metastases strongly express EGFR (91-100%) suggesting its role in penil cancer tumorogenesis[76-78]. Several anti-EGFR drugs were evaluated with mixed results. A retrospective analysis explored the role of EGFR-targeted therapies, including cetuximab, erlotinib and gefitinib in 24 SCPC metastatic patients. The median TTP and OS were 11.3 (1-40) and 29.6 (2-205) weeks, respectively. The OS was significantly shorter for patients with visceral or bone (24.7 *vs.* 49.9 weeks, p=0.013). Among 17 patients treated with cetuximab alone or in combination with cisplatin, there were 4 PR (23.5%), including two patients with apparently chemotherapy-resistant tumours. No clinical benefits were observed with gefitinib or erlotinib[129]. Necchi et al[130,131] evaluated the efficacy of panitumumab monotherapy at standard dose in pretreated unresectable or metastatic SCPC. Median PFS was 1.9 months [interquartile range (IQR), 0.9-3.0 months] and median OS was 9.5 months (IQR, 4.9-12.6). The presence of visceral metastases showed a trend for association with worse OS (p = 0.098)[130,131]. Recently, Rescigno et al[132] showed the efficacy and tolerability of a combination of cetuximab plus docetaxel in second line setting. An ongoing phase II study is evaluating the role of the Pan-HER inhibitor dacomitinib (PF-00299804) for locally advanced or metastatic SCCPC[133]. Another interesting phase 2 trial is evaluating the efficacy of afatinib in metastatic SCPC[134]. Antiangiogenic therapy has been demonstrated effective in the treatment of similar cancer types as lung and head and neck tumours. A retrospective case series[135] of six pretreated patients reported the efficacy of sunitinib or sorafenib in SCPC second line treatment. Finally, an omgoing phase II trial evaluating the combination of pazopanib and weekly paclitaxel in cisplatin pre-treated locally advanced or metastatic SCPC[136]. Overall, we have no clinical evidence to support the use of any TT in clinical practice.

Immunotherapy

The Program Death-1 (PD-1)/PD-1 ligand (PD-L1) axis has been demonstrated to play an important role in tumour immune escape, and immuno checkpoint inhibitors have shown stunning results in certain cancer types. In the last years, immunotherapy is emerging as a new therapeutic strategy to enhance the host immunity against cancer cells[137,138]. PD-L1 expression in SCPC was significantly associated with decreased cancer-specific survival, conversely the lack of primary tumour PD-L1 expression correlated with better clinical outcomes[139-141]. Moreover, a recently retrospective analysis, showed that 23 (62.2%) of 37 primary PC were positive for PD-L1 expression, with a strong positive correlation of PD-L1 expression in primary and metastatic samples[139]. Deng et al[142] confirmed that high PD-L1 expression in tumour cells was associated with poor prognosis. Notably, PD-L1 expression in tumour cells was significantly associated with the extent of TILs and CD8+ TILs[142]. These results were partially confirmed by Cocks et al[143] who showed no correlation between PD-L1 expression and patient age, tumour location, histologic subtype, tumour stage, anatomic depth of invasion, or tumour grade. On multivariable analyses of 200 primary PC a marginal expression pattern of PD-L1 was associated with absent lymph node metastases (OR 0.4) while diffuse expression was associated with poor survival (HR 2.58). These results were more prominent in the high-risk HPV negative subgroup (OR 0.25, HR 3.92)[144]. To date no immunotherapeutic agents are approved[145]. Several ongoing trails[146,147] are evaluating the role of different anti PD-1/PD-L1 in SCPC, alone or in combination with TT. Particularly, ongoing phase II trials is assessing the combination (NCT03333616) of the low dose of ipilimumab (1mg/kg) and the high dose of nivolumab (3 mg/kg)[148] and, a phase I (NCT02496208) evaluating this combination with the addition of the multityrosine kinase inhibitor (TKI) cabozantinib[149]. Targeting the HPV pathway with immunotherapeutic approaches, such as adoptive T cell therapy with tumour-infiltrating T cells selected for HPV E6 and E7 reactivity in conjunction with lymphocyte depleting chemotherapy and aldesleukin (a lymphokine) treatment, have demon-

strated encouraging efficacy in other HPV-related tumors, particularly cervical carcinoma. Based on that, an ongoing phase I trial (NCT02379520) is evaluating HPV-specific T cells in combination with the Nivolumab (anti-PD-1) in all HPV-related tumors, including SCPC[150].

Conclusions

SCPC is a rare tumor and, despite excellent outcomes in localized tumor, loco regional and metastatic disease remain a fatal disease with a shorter OS. The role of neoadjuvant or adjuvant chemotherapy is unclear, although several retrospective studies reported clinical benefits, particularly in clinical or pathological N2-3. In metastatic setting, a palliative chemotherapy can achieve a limited survival benefit and therefore, SCPC remains an orphan disease. The genomic landscape of SCPC is only partially understood, with a limited number of identified aberrant genes. With the advent of novel immunotherapy agents, the clinical need to personalize treatment has become more compelling. At the present time, there are no effective biomarkers that can be incorporated in the therapeutic algorithm, despite large research efforts. Due to its low incidence, particularly in developed countries, trials dedicated to penile carcinoma are difficult to conduct; therefore, an effort is required to centralize all patients, in view of an international collaborative group in order to upgrade the clinical and molecular research in this malignancy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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