The majority of penile squamous cell carcinomas (SCCs) show a conventional non-verruciform keratinizing squamous cell appearance (SCC of usual type). Histological variants included in the WHO histological classification of penile SCCs are: basaloid carcinoma, warty (condylomatous) carcinoma, verrucous carcinoma, papillary carcinoma, sarcomatoid carcinoma, mixed carcinomas and adenosquamous carcinomas. (1-7). In this presentation I would briefly discuss some of the less common histological variants already included in the WHO classification and also describe some additional histological variants such as pseudohyperplastic SCC, carcinoma cuniculatum and acantholytic SCC that were more recently reported (8-12). In addition, I would like to present some of our more recent work in the area of penile carcinoma that includes HPV-related studies, histopathological and immunohistochemical studies of precursor lesions and other studies related to prognosis.

1. Unusual And Recently Described Histopathological Variants

Pseudohyperplastic carcinomas

Pseudohyperplastic SCCs are non-verruciform low-grade tumors preferentially affecting the foreskin of older patients (8th decade) and strongly associated with lichen sclerosus (8, 9). There is extreme differentiation and in small biopsies the tumors mimic pseudoepitheliomatous hyperplasia. They are often multicentric and the second or third independent tumor may show some verrucous features. Grossly, they tend to be flat or slightly elevated measuring approximately 2 cm in greatest dimension. Characteristic microscopic features are infiltrative keratinizing nests of squamous cells with minimal atypia surrounded by a reactive stroma. This degree of differentiation is noted only in low-grade verruciform tumors such as verrucous or papillary cancers. The consistent association with lichen sclerosus suggests that this inflammatory condition may play a precancerous role. In a series of 10 cases, recurrence was noted in the glans of a patient 2 years after circumcision for a multicentric carcinoma of the foreskin. No metastases were found in any of these cases (8, 9).
**Acantholytic (pseudoglandular or adenoid) SCC**

This unusual variant of SCC is characterized by the presence of pseudoglandular spaces secondary to acantholysis (10). Patient's median age is 54 years. The tumors tend to be large, involve multiple penile anatomical compartments and deeply invade into erectile corpora. The pseudoglandular spaces contain acantholytic neoplastic keratinocytes sometimes admixed with keratin material and necrotic debris. CEA and mucin stains are negative. p63 and 34BetaE12 are diffusely positive. Acantholytic SCC usually harbor high grade foci, invade deep anatomical structures and therefore tend to be associated with a high incidence of regional metastasis and mortality. The differential diagnosis includes gland forming penile tumors (surface adenosquamous, mucoepidermoid and urethral adenocarcinomas) and angiosarcoma (2, 3).

**Papillary Carcinoma**

There is a group of low-grade papillomatous SCC of the penis, collectively designated as "verruciform", that are difficult to classify. A proposal of classification grouped these tumors in warty (condylomatous), verrucous and papillary carcinomas. Papillary carcinoma is an exophytic, slowly growing, low-grade SCC without HPV-related changes (2, 3, 5). It is usually located in the glans (51% of cases). Less frequently affects several anatomical compartments such as glans, coronal sulcus and foreskin (37%) or glans and sulcus (9%). Exclusive foreskin location is unusual (3%). Inguinal lymph node metastasis rate is low. Grossly, they are large (mean= 5.6 cm) white-gray exophytic and destructive lesion. The cut surface usually shows a pearly white tumor with a serrated papillomatous surface and ill defined deep borders. Microscopically, the appearance is that of a well-differentiated papillary squamous neoplasm with acanthosis and hyperkeratosis. The papillae show prominent fibrovascular cores and arborescent complex appearance. Koilocytotic-like changes are not seen. The interface between tumor and stroma is infiltrative. The majority of the tumors are well to moderately differentiated. Most lesions invade corpus spongiosum and/or dartos (77% cases). Vascular and perineural invasion are unusual. Papillary carcinomas have a good prognosis with nodal metastases seen in approximately 30% of patients. Mortality is low. The differential diagnosis includes verrucous carcinoma, carcinoma cuniculatum and warty carcinoma. Verrucous carcinomas share some architectural (papillary surface) and cytological (degree of differentiation and lack of koilocytosis) features with papillary carcinoma; however the deep border of verrucous carcinoma is classically broad and pushing compared to the jagged deep border of papillary carcinoma. In addition, papillary carcinoma tends to be slightly less differentiated that verrucous carcinoma, especially in deeper portions of the neoplasm. Carcinoma cuniculatum is a hybrid variant of verrucous carcinoma with a classical gross appearance. Warty carcinomas are HPV-related tumors that can be distinguished from papillary carcinomas by the marked pleomorphism and koilocytosis.
Carcinoma cuniculatum

This unusual tumor was originally described by Ayrd et al in the plantar skin as a peculiar variant of verrucous carcinoma characterized by a deep burrowing growth pattern mimicking rabbit burrows (cuniculum) (11). Rare cases have been reported in the penis (12). The mean patients’ age is 77 years. Grossly, the tumors are large, papillomatous lesions with cobblestone appearance, usually affecting the glans and extending to the coronal sulcus and foreskin. Cut surface shows the hallmark of the lesion represented by deep and narrow, complex tumor invaginations that connect to the surface through sinus tracts. Microscopically, the bulk of the lesion has features of a verrucous carcinoma (extremely well differentiated with bulbous deep borders) usually associated with a minor component that is more infiltrative and less differentiated. In most cases, therefore, it represents a hybrid verrucous/usual SCC with a peculiar growth pattern. The deep invaginations form interanastomosing channels and pseudocystic structures that are lined by well differentiated carcinoma and filled with keratin material. Carcinoma cuniculatum appears to have a good prognosis. None of the reported cases metastasized (12).

Mixed squamous cell carcinomas

Squamous cell carcinomas of the usual type can be found in association with any other subtypes of penile cancer. Mixed carcinomas account for about 25% of penile SCCs. The classical example is that of a verrucous carcinoma with higher grade foci consisting of usual SCC, so-called hybrid carcinoma (13). The metastatic potential of these hybrid tumors is related to the grade and depth of invasion of the non-verrucous component (13). Another interesting mixed tumor is the warty-basaloid SCC, where an exophytic warty tumor is associated with a deeply invasive basaloid carcinoma (14). Clinical behavior is related to the basaloid component, with a high rate of regional metastasis.

2. Penile Intraepithelial Neoplasia (PeIN)

Invasive squamous cell carcinomas are thought to be preceded by precursor lesions, namely penile intraepithelial neoplasms (PeIN). In keeping with the notion of a bimodal pathway of carcinogenesis in penile location, precursor lesions can be broadly classified in two main groups: HPV-related and HPV-unrelated variants. We have recently proposed a slightly modified nomenclature for penile preinvasive lesions (15). The term penile intraepithelial neoplasia (PeIN) is preferred over old terms such as squamous intraepithelial lesion (SIL), erythroplasia of Queyrat and Bowen’s disease. These latter two terms are synonymous with carcinoma in situ and have been used for lesions in the glans (erythroplasia of Queyrat) and skin of the shaft (Bowen’s disease). PeIN can be classified in differentiated/simplex (HPV-unrelated) and HPV-related variants. The latter includes warty, basaloid and mixed warty/basaloid. PeIN may be solitary or multifocal, and tend to be associated with infiltrating SCCs, in about two thirds of cases. In our experience, of these cases, approximately 65% tend to be associated with differentiated PeIN and 35% with warty/basaloid PeIN. Differentiated PeIN affects older patients, it usually arises in the setting of a chronic scarring inflammatory dermatosis and it is more frequently located in the
foreskin when compared with HPV-related variants. The latter affects younger patients and are usually more centrally in the glans and perimeatal region. The gross appearance of PeIN is heterogeneous and does not allow to distinguish between the different types. Lesions vary from flat to slightly elevated, pearly white or moist erythematous, dark brown or black, macules, papules, or plaques. The contours may be sharp or subtle and irregular. Microscopically, differentiated (simplex) PeIN is characterized by a thickened epithelium, usually associated with elongated and anastomosing rete ridges, subtle abnormal maturation (enlarged keratinocytes with abundant eosinophilic cytoplasm), whorling and keratin pearl formation (usually in deep rete ridges), prominent intercellular bridges (spongiosis and sometimes acantholysis) and atypical basal cells with hyperchromatic nuclei (15). Parakeratosis is frequent. A low power, the atypia seems to be present only in lower levels of the epidermis; however, at higher power, it is clearer that there is subtle but abnormal maturation in all levels of the epithelium. Despite the subtle changes, we believe that differentiated PeIN represents a high grade (although differentiated) lesion that may evolve to frank invasive carcinoma without showing more significant atypias (15). It is not surprising that the precursor lesions of well-differentiated invasive tumors show such a high degree of differentiation. It is important to recognize this lesion because it appears to be the most frequent precursor lesion of penile carcinomas, especially the keratinizing and well differentiated variants. A preferential association was seen between lichen sclerosus and differentiated PeIN when compared with warty/basaloid variants (16, 17). It is therefore important to keep a high level of suspicion when dealing with hyperkeratotic/hyperplastic lesions with subtle keratinocytic atypia arising in the setting of long standing lichen sclerosus (16, 17).

The second major group of PeIN corresponds to the HPV-related lesions such as warty, basaloid and mixed PeIN. In basaloid PeIN, the epithelium is replaced by a monotonous population of small immature cells with high nuclear/cytoplasmic ratio (15). Apoptosis and mitotic figures are numerous. In warty PeIN, the involved epithelium has an undulating/spiking surface with atypical parakeratosis. There is striking cellular pleomorphism and koilocytosis (multinucleation, nuclei with irregular contours, perinuclear halo and dyskeratosis). Mitosis tend to be numerous. Frequently, lesions show overlapping features of both, namely mixed warty/basaloid PeIN. These mixed lesions tend to have a spiking surface with koilocytic changes while the lower half of the epithelium is predominantly composed of small basaloid cells. Basaloid and warty PeIN can be divided in low grade and high grade lesions when the atypical cells occupy less than half of more than half of the epithelial thickness respectively. Most of the warty and basaloid PeIN will fall within the high grade category. Full thickness atypia of the epithelium equals carcinoma in situ. p16 is usually over-expressed in warty and basaloid PeIN and negative in differentiated PeIN (15).

3. HPV and p16 in Penile Carcinoma

The overall prevalence of HPV-DNA detection in penile cancers is approximately 40-50% and this figure is very similar to that detected in vulvar carcinomas (18-20). No significant differences in HPV prevalence were seen when comparing cases from USA and Paraguay (18). Although HPV reveals a remarkable plurality of different genotypes, only a limited
number are associated with penile carcinomas. High-risk HPV 16 is by far the most frequently type associated with penile cancer, followed by HPV 18. Tumor types that are significantly related to HPV include the warty and basaloid variants. In a recent study it was found that the “basaloid” cell (small, round blue cell) is the single most reliable morphological feature that correlates with the presence of HPV in a particular tumor. At a molecular level, p16INK4a appears to be a specific marker for cells that express the viral E6-E7 oncogenes (21, 22). Since expression of p16INK4a underlies a negative feedback control through pRB, the enhanced expression of p16 is probably due to reduced or lost pRB function. Binding of HPV-E7 oncoprotein to pRb causes degradation of pRb with consequent loss of Rb-tumour suppressor function and p16 overexpression, which can be demonstrated immunohistochemically. We recently found a significant over-expression of p16 in warty and warty-basaloid tumors when compared with HPV-unrelated variants of verruciform lesions (23). Interestingly, the precursor lesions of HPV-related tumors (warty and warty/basaloid PeIN) were strongly positive for p16 while the precursor lesions of HPV-unrelated tumors (differentiated PeIN and lichen sclerosus) did not express p16 further supporting the concept of a bimodal pathway of tumor progression.

It is important to recognize that the majority (approximately 55-60%) of penile cancers appear to be HPV-unrelated. Very little is known about the molecular pathway involved in the carcinogenesis of HPV-unrelated tumors and this is an area that needs to be investigated.

4. Prognostic Index

Considering the high morbidity of groin dissection, there is need to find a method to better select potential candidates that could benefit from this procedure. Sentinel node biopsy (especially the dynamic method) appears as a promising methodology to identify early metastatic foci. The high rate of recurrences rate (in most studies), lack of multicenter reproduction, and cost of dynamic sentinel node biopsies preclude their routine implementation in developing countries and other approaches are necessary. Because histologic grade, depth of tumor infiltration, and perineural invasion (PNI) are considered among the most important pathologic prognostic parameters in penile cancer (24-28), we devised a Prognostic Index combining these 3 factors (29). The Prognostic Index (ranging from 2 to 7) consists in the addition of numerical values given to histologic grade (1 to 3), deepest anatomic level involved by cancer (1 to 3), and presence of PNI (0 or 1). Histologic grades were defined as follows: grade 1, carcinomas with minimal to no atypias; grade 3, tumors showing any proportion of anaplastic cells; and grade 2, the remainder tumors. The anatomic levels and their numerical values were: in glans, lamina propria, 1; corpus spongiosum, 2; and corpus cavernosum, 3. In foreskin they were: lamina propria, 1; dartos, 2; and skin, 3. PNI was evaluated as follows: absence of PNI, 0; presence of PNI, 1. Prognostic Index scores were found as the best predictors of inguinal node metastasis and patients' survival in a recent study (29). Inguinal node dissections might not be necessary for patients with low indices (2 and 3). Nodal dissections might be formally indicated for high-grade indexes (5 to 7). Patients with index 4 should be individually assessed for nodal dissection. Prognostic Index might represent a useful pathologic guide to the clinicians in the often difficult decision whether to perform or not an inguinal dissection.
REFERENCES


