**MATERIAL & METHODS**

**Case Selection, Tissue Microarray Building, and Morphologic Evaluation**

The present study includes tissue samples from 112 patients with invasive squamous cell carcinomas of the penis diagnosed at the Instituto de Patología e Investigación (Asunción, Paraguay) between 2000 and 2011. From each case, 1 to 4 formalin-fixed, paraffin-embedded blocks were selected, based on tumor tissue availability. Using a previously described procedure [[1](#_ENREF_1)], 4 tissue microarrays (TMAs) were built at the Johns Hopkins TMA Lab Core (Baltimore, MD). Three tissue cores of 1 mm each were obtained per block, giving a representation of 3–12 TMA spots per case.

Histologic subtyping was carried out in whole tissue sections using the morphologic criteria presented in the *Atlas of Tumor Pathology* of the Armed Forces Institute of Pathology [[2](#_ENREF_2)]. Histologic grading was carried out spot by spot using previously published and validated criteria [[3](#_ENREF_3)]; for statistical analysis, the highest grade at the TMA spots was assigned as the histologic grade of the case.

**Immunohistochemistry and Scoring System**

Each TMA spot was scanned using the APERIO system (Aperio Technologies, Inc., Vista, CA) and uploaded to TMAJ, an open-source platform for online evaluation of TMA images (available at http://tmaj.pathology.jhmi.edu). Images were scanned at a 20x resolution, yielding an image scale of 2.65 microns/mm.

**REFERENCES**

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3. Chaux A, Torres J, Pfannl R, Barreto J, Rodriguez I, Velazquez EF, et al. Histologic grade in penile squamous cell carcinoma: Visual estimation versus digital measurement of proportions of grades, adverse prognosis with any proportion of grade 3 and correlation of a Gleason-like system with nodal metastasis. Am J Surg Pathol. 2009;33(7):1042-8.