

# Concordance of lymphovascular invasion diagnosed in penile carcinoma with and without the immunohistochemical markers ERG and CD31

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**Summary.** Lymphovascular invasion (LVI) is an independent predictor of metastatic lymph node disease in penile carcinoma and is one factor used to guide clinical management. The presence of LVI with and without the use of the endothelial immunohistochemical (IHC) markers, ERG and CD31, was retrospectively assessed in 46 penectomy cases containing invasive penile carcinoma (43 squamous cell carcinoma and 3 non-squamous cell carcinoma). Concordance for the detection of LVI between the original report, upon pathology review, and with the use of IHC was determined and histologic pitfalls were identified. For penile squamous cell carcinoma, LVI was diagnosed in 27.9% of tumors in the original reports, 16.3% upon pathology review, and in 16.3% with use of ERG and CD31. Concordance of LVI identification in the original report compared to IHC was 74.4% while concordance of review compared to IHC was 95.3%. Using IHC data as the reference, false positive LVI diagnoses were more common in the original report than false negatives. Histologic mimickers of LVI including involvement of the penile corpora cavernosum or spongiosum vasculature, seromucinous colonization, and a nested pattern of tumor invasion were identified. We demonstrated that it was not uncommon for LVI in penile carcinoma to be overdiagnosed or underdiagnosed. The use of endothelial IHC markers, such as ERG or CD31, or additional pathology consultation is recommended for penectomy cases in

which LVI is difficult to histologically discern.

**Key words:** Penile, Carcinoma, Lymphovascular, Immunohistochemistry, ERG

## Introduction

The incidence of penile carcinoma is rare, particularly in the developed world, with the most common type being squamous cell carcinoma (SCC). Penile carcinoma has been found to have possible associations with human papillomavirus infection, smoking, and chronic inflammation (Chaux and Cubilla, 2012). The tumor has a predictable pattern of local invasion which first involves the subepithelial tissue and then extends through the corpus spongiosum and into deeper structures including the corpus cavernosum and penile urethra (Chaux and Cubilla, 2012). Treatment encompasses a spectrum of clinical management such as laser therapy, wide local excision, and partial or total penectomy (Clark and Spiess, 2013).

The extent of therapeutic intervention is influenced by pathologic staging. The pT designation of a penile carcinoma is based upon tumor grade, lymphovascular invasion (LVI) and depth of invasion. Inguinal lymph node metastasis is the most important prognostic factor in patients with penile carcinoma, and LVI has been identified as an independent predictor of lymph node involvement (Chaux and Cubilla, 2012). Because of this, the presence or absence of LVI directly affects clinical management of the disease. Tumor with LVI alone is staged as pT1b, while invasion of the corpora is pT2

(Edge et al., 2010). According to the practice guidelines by the National Comprehensive Cancer Network, if LVI is present, a sentinel node biopsy or inguinal lymph node dissection is indicated (Clark and Spiess, 2013). The European Association of Urology advises elective bilateral inguinal lymphadenectomy in clinically node negative patients with LVI (Graafland et al., 2010). The morbidity of sentinel lymph node biopsy is relatively low; however, patients who undergo complete inguinal lymph node dissection have a 24-87% risk of post-operative complications and a 3% mortality rate (Heyns et al., 2010).

Although LVI is an important risk factor in penile cancer, multiple investigations have noted that the histological identification of LVI can be challenging (Graafland et al., 2010; Chaux and Cubilla, 2012). To our knowledge, no publications have assessed the interobserver variability of identification of LVI or compared standard histologic assessment to use of immunohistochemical (IHC) markers in penile carcinoma. The goal of this study was to evaluate the reproducibility of identifying LVI in penectomy specimens with and without the use of endothelial IHC markers and to identify histologic pitfalls leading to misinterpretation.

## Materials and methods

All penectomy cases with invasive penile carcinoma performed between January 2006 and November 2012 at The Ohio State University Wexner Medical Center were retrospectively obtained. Cases were signed out in a subspecialized academic pathology practice. The presence or absence of LVI per the original pathology report, case pathologist, surgeon, tumor type, and tumor stage were recorded. All hematoxylin and eosin (H&E) slides were reviewed by a genitourinary surgical pathologist to assess for LVI, blinded to lymph node status and case pathologist. Specimens had been grossed via standardized methodology, described briefly as follows (Ebel et al., 2013). The glans was bivalved followed by bread-loafing or bivalving the penile shaft. At least one section per centimeter of tumor and three cross sections including tumor with the greatest depth of invasion were submitted.

A pilot study was done to identify which IHC markers would best highlight endothelial cells in these specimens. A single representative block containing invasive penile carcinoma was obtained and processed with standard H&E staining, and a corresponding slide was stained with one of the following IHC endothelial markers: ERG (Biocare Medical, Concord, CA, dilution 1:50), CD31(JC/70A) (Dako, Carpinteria, CA, dilution 1:400), CD34 (Novocastra, Buffalo Grove, IL, dilution 1:400), D2-40 (Dako, Carpinteria, CA, dilution 1:150), Factor VIIIIR (Dako, Carpinteria, CA, dilution 1:120), CD141 (Novocastra, Buffalo Grove, IL, dilution 1:200), and WT1 (Dako, Carpinteria, CA, dilution 1:500). One to 3 cases were used for each antibody. Of these, ERG

and CD31 displayed the least background staining with the clearest positivity in endothelial nuclei and cytoplasm and membrane, respectively.

ERG and CD31 were subsequently used for the remainder of the experiments. A single representative block was chosen from each penectomy case. The block corresponding to the slide noted to contain LVI by the original pathologist was selected, if applicable. If no slides were marked as containing LVI, the block corresponding to the slide with areas most suspicious for LVI was chosen. If no area appeared suspicious, the block with the deepest tumor invasion was utilized. 4 micrometer sections from each case were oven dried at 55°C for 3 hours, loaded on a Leica-Bond Autostainer, and subjected to heat-induced epitope retrieval in Bond Epitope Retrieval Solution 1 at pH 6.0 for 25 minutes. The slides were then incubated with a primary monoclonal antibody specific for ERG (15 minutes) or CD31 (30 minutes). Bond Polymer Refine Detection kit was applied and the sections were counterstained with hematoxylin. Each case was analyzed for nuclear staining if ERG was used and cytoplasmic and membranous staining for CD31. Colon and placenta were used as positive control and tissue lacking the primary antibody was used as a negative control.

Concordance for the detection of LVI between the original report and pathology review was determined. ERG and CD31 immunostains were then assessed for LVI, in conjunction with corresponding H&E slides to compare the concordance for the detection of LVI between the original report vs IHC and pathology review vs IHC. Tumors of non-SCC histology were described but were excluded from concordance calculations.

## Results

46 cases of penectomies for invasive penile carcinoma were identified retrospectively. The resections were performed by 8 surgeons and were signed out by 8 pathologists. Of the pathologists, 3 were genitourinary fellowship trained and signed out 24 of the cases (52.2%). The age range of the cohort was 42-91 years (mean 65). Per pathology report, tumor sizes ranged from 1.6-16.2 cm (mean 5.2).

## SCC

43 of the 46 cases were diagnosed as SCC (93.5%) with pT and pN classification at the time of resection shown in Table 1. A summary of the results comparing the interpretation of LVI in the pathology report, H&E review, and with the use of IHC for the 43 cases of SCC is shown in Table 2.

Data analysis comparing the identification of LVI on the original report with the pathology review revealed an overall concordance of 79.1% (34/43) with a positive percent agreement of 41.7% (5/12) and a negative percent agreement of 93.5% (29/31). Of the 43 cases, 5 cases were reported to be positive for LVI by both report

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and review (Fig. 1A1) and 29 cases were found to be negative for LVI by both report and review. Of the 9 discordant cases, there were 4 in which LVI was initially found on original report, but on review of the original H&E slides a nested pattern of tumor invasion was seen without LVI (Fig. 1B1). An additional 3 cases had LVI diagnosed in the original report, but on pathology review, invasion of the large anastomosing vascular structures of the penile corpora cavernosum or spongiosum was seen, with no small vessel invasion detected (Fig. 1C1). In these cases, the tumors had continuous involvement of the large vascular channels, with most of the vascular structures entirely filled with elongated cords of tumor. In 2 cases, LVI was not diagnosed on original report but was identified on review (Fig. 1D1).

In comparing results of the original report to the use of IHC, there was an overall concordance of 74.4% (32/43) with a positive percent agreement of 33.3% (4/12) and a negative percent agreement of 90.3% (28/31). Of the 43 cases reviewed, 4 cases were found to contain LVI on both original report and with use of IHC (Fig. 1A2) and 28 cases were found to be negative for LVI by both methods of identification. Of the 11 discordant cases, 3 of these cases had corpora vascular involvement mimicking LVI (originally reported to be positive for LVI). Endothelial vascular markers highlighted the architecture of the anastomosing blood vessels, inconsistent with LVI (Fig. 1C2). 5 cases with a nested pattern of tumor invasion were also originally reported to contain LVI, but use of IHC failed to corroborate this interpretation (Fig. 1B2). Of the remaining discordant cases, 3 cases were originally reported to be negative for LVI but then were found to be positive on IHC (Fig. 1D2).

Analysis of concordance between the pathology review and use of IHC showed an overall 95.3% (41/43) concordance rate with an 85.7% (6/7) positive percent agreement and a 97.2% (35/36) negative percent agreement. 6 cases were found to be positive for LVI on

both review and by IHC, and 35 cases were found to be negative for LVI on both. 1 discordant case was found to be positive on pathology review but negative with use of IHC. In another, LVI was seen only on IHC slides; this focus was not present in the H&E (Fig. 1E1).

5 cases had lymph node dissections performed with the penectomy specimens with an additional 13 having dissections performed at our hospital within 4 months of the penectomy (combined pN: 25 pNX, 8 pN0, 1 pN1, 2 pN2, 7 pN3). In patients that did not undergo lymph node dissection either at penectomy or subsequently (pNX), the frequency of detected LVI was lower upon review (16.0%, 4/25) and by IHC (16.0%, 4/25) versus original report (24.0%, 6/25). In patients that underwent a lymph node dissection after the penectomy and had positive lymph nodes (pNX at penectomy and pN+ within 4 months of the penectomy), the frequency of detected LVI was higher upon review (50.0%, 2/4) and by IHC (50.0%, 2/4) versus original report (25.0%, 1/4).

### Non-SCC

In addition to the 43 cases of penile SCC, 2 cases contained urothelial carcinoma and 1 case was spindle cell carcinoma. In 1 of these, there was discordance between LVI identified in the pathology report and review but negative with the use of IHC (Fig. 1F1). Re-review of the H&E revealed the presence of seromucinous glands colonized by urothelial carcinoma mimicking LVI (Fig. 1F2). In the other case of urothelial carcinoma, invasion into the vascular structures of the penis was originally diagnosed as LVI on report but was identified as corpora invasion on review and with use of IHC.

### Discussion

LVI is an important prognostic factor in carcinoma of the penis and cancers of other organs; however, challenges such as retraction artifact and stromal compression can give a false histologic impression of the presence of LVI (Mohammad et al., 2007). In addition, there can be significant interobserver variability between pathologists' interpretation of LVI (Fan et al., 2010; Graafland et al., 2010). Superficial penile biopsies are often used to evaluate penile tumors. However,

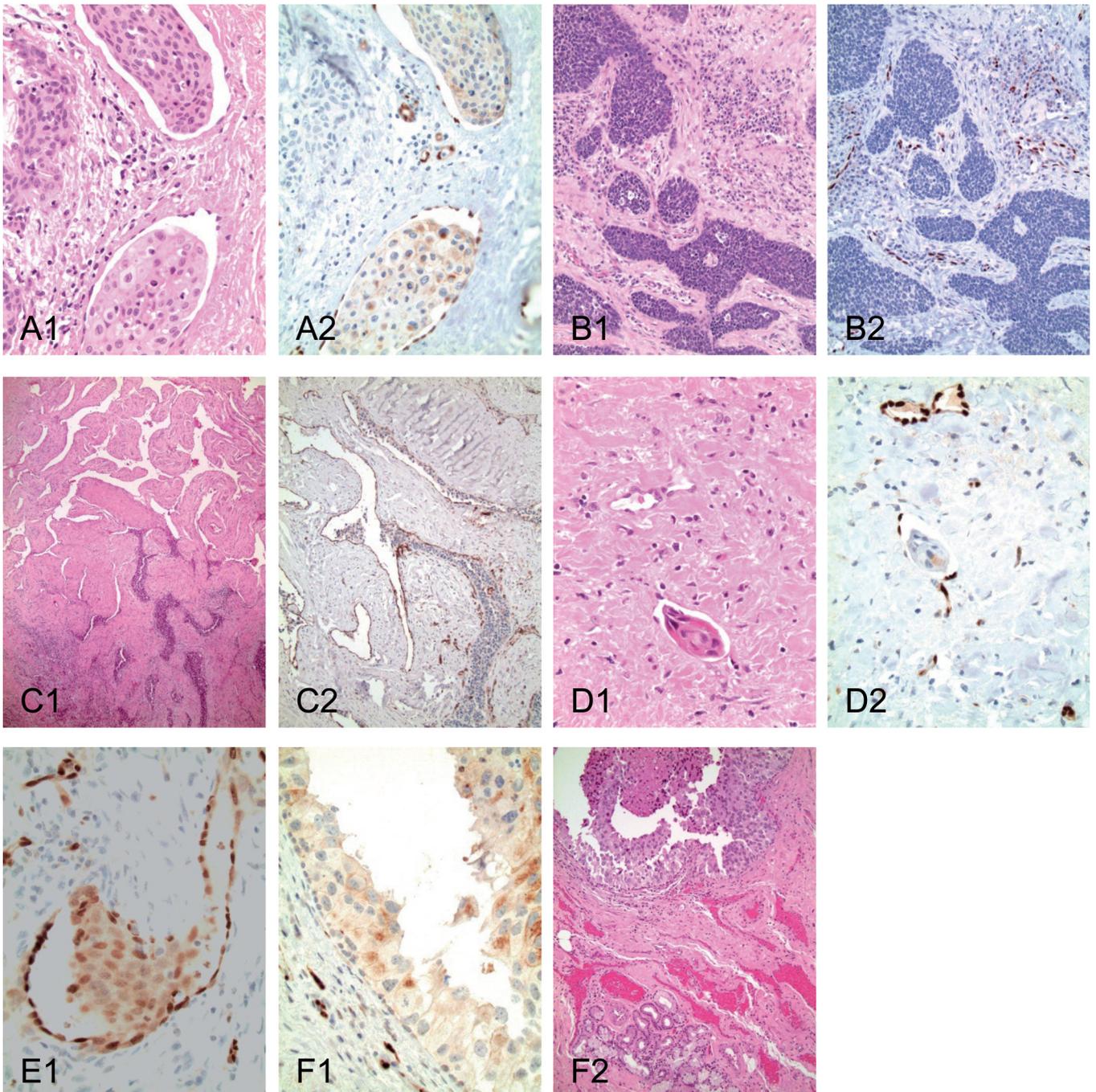
**Table 1.** pT, pN, and LVI status according to the original pathology report for penectomy resections containing SCC.

	%	LVI +, n (%)	LVI -, n (%)
<b>pT</b>			
pT1a (n=6)	14.0	0	6 (100.0%)
pT2 (n=20)	46.5	5 (25.0%)	15 (75.0%)
pT3 (n=15)	34.9	5 (33.3%)	10 (66.6%)
pT4 (n=1)	2.3	1 (100.0%)	0
Unspecified* (n=1)	2.3	1 (100.0%)	0
<b>pN</b>			
pNX (n=38)	88.4	9 (23.7%)	29 (76.3%)
pN1 (n=2)	4.7	1 (50.0%)	1 (50.0%)
pN2 (n=1)	2.3	0	1 (100.0%)
pN3 (n=2)	4.7	2 (100.0%)	0

\*pT2 on pathology review.

**Table 2.** Comparison of LVI interpretation in the pathology report, H&E review, and with use of IHC for SCC in penectomy specimens.

LVI Report/Review/IHC	n (%)
+/+/+	4 (9.3%)
+/+/-	1 (2.3%)
+/-/-	7 (16.3%)
-/+/+	2 (4.7%)
-/-/+	1 (2.3%)
-/-/-	28 (65.1%)

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**Fig. 1.** Representative photomicrographs. **A1.** H&E photomicrograph showing SCC within a lymphovascular space in a case with LVI identified on original report, review, and IHC. **A2.** ERG IHC from the case shown in A1 demonstrates cells with strong positive nuclear reactivity surrounding the tumor emboli. **B1.** H&E photomicrograph showing a nested pattern of invasion of SCC mimicking LVI in a case with LVI identified on original report, but not seen on review or IHC. **B2.** ERG IHC from the case shown in B1 is negative surrounding the tumor, providing evidence that the nests of tumor are not within vessels. **C1.** H&E of SCC demonstrating invasion of the anastomosing vascular channels of the penile corpora cavernosum, which was mistaken for LVI in the original report. The tumor completely fills the large anastomosing vessels in a contiguous pattern. **C2.** CD31 IHC highlights the endothelium of the corpora cavernosum which contains tumor in the lumen in the case in C1. **D1.** H&E photomicrograph of a small foci of SCC within a lymphovascular space in a specimen in which LVI was not identified in the original report but was seen on review and IHC. **D2.** ERG IHC of the case in D1 identifies cells with nuclear positivity in the endothelium of this vessel surrounding the tumor. **E1.** ERG IHC of SCC demonstrating strong nuclear reactivity in the endothelial cells surrounding a cluster of tumor. This focus was only present on the immunostain and was not seen in the original H&E. **F1.** ERG IHC of the case seen in **F2** demonstrates negative nuclear reactivity of the flattened cells surrounding the tumor. **F2.** H&E image showing seromucinous colonization by urothelial carcinoma, which was mistaken for LVI in a patient with LVI identified on original report and review, but not by IHC shown in F1. A1, A2, D1, D2, E1, F1, x 40; B1, B2, x 20; C1, x 4; C2, F2, x 10

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Velazquez et al. (2004) described only 1 of 9 cases to have concordant diagnoses of LVI between biopsy and penectomy, although they did not comment if over or underdiagnosis contributed to the discordance. Previous investigations examining tumor characteristics in penectomy patients with penile carcinoma without immunostains describe the presence of LVI in 31-44% of cases (Slaton et al., 2001; Ficarra et al., 2006; Novara et al., 2008; Cubilla, 2009). We assessed the presence of LVI in invasive penile carcinoma within penectomy specimens with the majority of cases being SCC. To our knowledge, this is the first study to assess LVI with or without endothelial IHC markers in penectomy specimens. Our research showed that the over and underdiagnosis of LVI in penile carcinoma was not uncommon despite LVI being an important factor in clinical management.

The use of endothelial specific IHC markers could help accurately identify LVI in cases that are histologically difficult to interpret. After testing several endothelial antibodies in penectomy specimens, ERG and CD31 were selected due to their broad reactivity in both lymphatic and blood vessels and low background, producing strong staining that was easy to interpret. Additionally, we found having 1 nuclear and 1 non-nuclear marker desirable. Comparing LVI in penile SCC diagnosed by IHC to the identification of LVI in the original report, there was a 74.4% concordance with most discrepancies due to overdiagnosis. The interpretation of LVI using IHC had similar results to the histology-only review, yielding a 95.3% concordance.

In our investigation, we identified several histologic findings that mimicked LVI. A nested pattern of SCC invasion seen on H&E resembled LVI, but use of IHC showed the malignant cells were not present in an endothelial-lined space. An additional mimicker of LVI was tumor invasion into the vascular channels of the corpora spongiosum and cavernosum that was originally identified as small vessel invasion. The presence of endothelium surrounding tumor is widely accepted as a marker of LVI for many tissue types; however, in penile carcinoma it is important to make a distinction between LVI and invasion into the anastomosing vascular structures of the penile corpora cavernosum and spongiosum. A tumor with LVI alone is staged as pT1b, while invasion of the corpora is pT2. Tumors with invasion of the vasculature of the corpora cavernosum or spongiosum had continuous involvement of the large vascular channels. Many of the vascular structures were entirely filled with thick cords of tumor rather than the small, disconnected and dispersed emboli seen in cases with only LVI. Additionally, seromucinous colonization of the penile urethra with urothelial carcinoma was also a cause of LVI overdiagnosis, re-diagnosed after evaluating the immunostains. Other histologic findings which have been described to lead to false identification of LVI include retraction artifact around tumor sheets, stromal compression around tumor foci, and dis-

placement of benign or malignant cells in a vascular lumen (Mohammed et al., 2007; Kryvenko and Epstein, 2012; Kojima et al., 2013). While less common, we did detect underdiagnosis of LVI. It has been reported that with small tumor emboli, LVI may be missed due to the region not being on the sample slide, and this occurred in 1 case in our study (Naumann et al., 2008). Other factors noted in the literature to contribute to underdiagnosis of LVI is that the thin endothelial cell lining may be difficult to appreciate, particularly if the lumen is obliterated by tumor, and that tumor nests may obscure intermixed small regions of LVI (Aranout-Alakarain et al., 2007; O'Donnell et al., 2008; Imamura et al., 2012).

Prior authors have noted the challenging nature of diagnosing LVI in SCC in other organ systems as well. For example, in oral SCC, a high incidence of false positive and false negative LVI reporting was found (Kurtz et al., 2005). However, in cervical SCC, one study found that out of 41 cases, there was a 90% agreement regarding the presence of LVI on review (Noviello et al., 2008). The use of IHC to improve identification of LVI has not been previously assessed in penile carcinoma but has been examined in non-penile SCC. Research examining SCC of the oropharynx, esophagus, and vulva found that the use of IHC (CD31, CD34, D2-40, and/or cytokeratin) increased the percentage of cases with LVI detected from 15-41% to 42-69% (Kurtz et al., 2005; O'Donnell et al., 2008; Braun et al., 2009; Imamura et al., 2012). The use of IHC to detect LVI has been extensively examined in other types of carcinoma. Multiple studies have found that the use of IHC increased the number of breast tumor cases with LVI detected (Mohammed et al., 2007; Arnaout-Alkarain et al., 2007; de Mascarel et al., 2009). However, another publication analyzing IHC in colorectal cancer had a decrease in LVI from 43% to 29%, similar to our study (Kim et al., 2013).

In conclusion, we found that it was not uncommon for LVI in penile carcinoma to be over or underdiagnosed. A challenge unique to the identification of LVI in penile carcinoma is the lack of experience in diagnosing this organ for most pathologists due to the low incidence of penile carcinoma. Additionally, histologic mimickers of LVI such as involvement of the penile corpora cavernosum or spongiosum vasculature, seromucinous colonization, and a nested pattern of tumor invasion may lead to incorrect interpretation. For cases in which LVI is difficult to histologically discern, we recommend the use of endothelial IHC markers and/or additional pathology consultation.

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*Conflicts of interest statement.* We declare that we have no conflict of interest.

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