



Original contribution

PAX8 positivity in nested variant of urothelial carcinoma: a potential diagnostic pitfall[☆]

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Summary Nested variant of urothelial carcinoma is a rare variant of urothelial carcinoma morphologically characterized by infiltrative nests of cytologically bland urothelial cells. It is widely recognized that nested variant of urothelial carcinoma can closely mimic von Brunn nests. However, nested variant of urothelial carcinoma with tubule formation can also resemble nephrogenic adenoma, where immunohistochemical positivity for PAX8 has been used to establish the diagnosis of nephrogenic adenoma. Following anecdotal examples of PAX8 positive nested variant of urothelial carcinoma, we formally evaluated 23 cases of nested variant of urothelial carcinoma from 2011 to 2018. Cases were collected from our institution and evaluated for their architectural pattern and PAX8 expression. Except for 1 case from the renal pelvis, cases were located in the bladder. The majority (14/23 [61%]) showed solid nests with at least focal tubular differentiation. PAX8 immunoreactivity was strong (3+) in 7 (30%), moderate (2+) in 6 (26%), and negative in 10 (44%) cases. Four (57%) of the cases with strong expression and 3 (50%) of those with moderate staining showed diffuse immunoreactivity. Moderate-strong immunoreactivity was seen in 4/6 (66.7%) cases with solid nests, 8/14 (57.1%) with solid nests and tubules, and 1/3 (33.3%) with large nests. In conclusion, PAX8 can be positive in a significant proportion of nested variant of urothelial carcinoma cases, and recognition of this finding is important to avoid misdiagnosis of nested variant of urothelial carcinoma as nephrogenic adenoma based on PAX8 expression, particularly in cases with tubular differentiation and limited sampling.

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1. Introduction

In 1979, Stern described an “unusual bladder tumor consisting of proliferating benign Brunn nests” [1], and in 1992, Murphy and Deana recognized that despite the initial

impression of a benign lesion resembling a proliferation of Brunn's nests, these lesions were carcinomas with an aggressive course and coined the name “nested variant of transitional cell carcinoma” [2].

The nests in nested variant of urothelial carcinoma can be solid or solid with tubular differentiation. A large nested pattern has also been described [3]. Common to all patterns is low-grade cytology with occasional nuclear enlargement often suggesting a benign process. Subsequently, the morphologic differential diagnosis of nested variant of urothelial carcinoma includes mostly benign entities such as von Brunn nests,

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Case	Age	Sex	PAX8	PAX8, intensity	PAX8, extent (%)	Location	Architectural pattern
1	57	M	Negative	-	-	Bladder	Solid nests
2	44	M	Positive	Strong	80	Bladder	Solid nests
3	58	F	Negative	-	-	Bladder	Solid and tubular
4	77	M	Negative	-	-	Bladder	Solid and tubular
5	63	M	Negative	-	-	Bladder	Solid nests
6	71	M	Negative	-	-	Bladder	Solid and tubular
7	59	M	Positive	Moderate	70	Bladder	Solid and tubular
8	74	M	Negative	-	-	Bladder	Large nested
9	63	F	Negative	-	-	Bladder	Solid and tubular
10	68	F	Negative	-	-	Bladder	Large nested
11	67	F	Positive	Moderate	60	Bladder	Solid and tubular
12	63	F	Positive	Moderate	70	Bladder	Solid nests
13	57	M	Positive	Moderate	30	Bladder	Solid and tubular
14	63	F	Negative	-	-	Bladder	Solid and tubular
15	66	M	Negative	-	-	Bladder	Solid and tubular
16	72	M	Positive	Strong	40	Bladder	Solid and tubular
17	58	M	Positive	Strong	40	Bladder	Large nested
18	61	F	Positive	Moderate	40	Bladder	Solid nests
19	74	F	Positive	Strong	100	Bladder	Solid and tubular
20	65	M	Positive	Strong	90	Bladder	Solid nests
21	58	F	Positive	Moderate	40	Bladder	Solid and tubular
22	55	F	Positive	Strong	40	Bladder	Solid and tubular
23	73	F	Positive	Strong	100	Renal pelvis	Solid and tubular

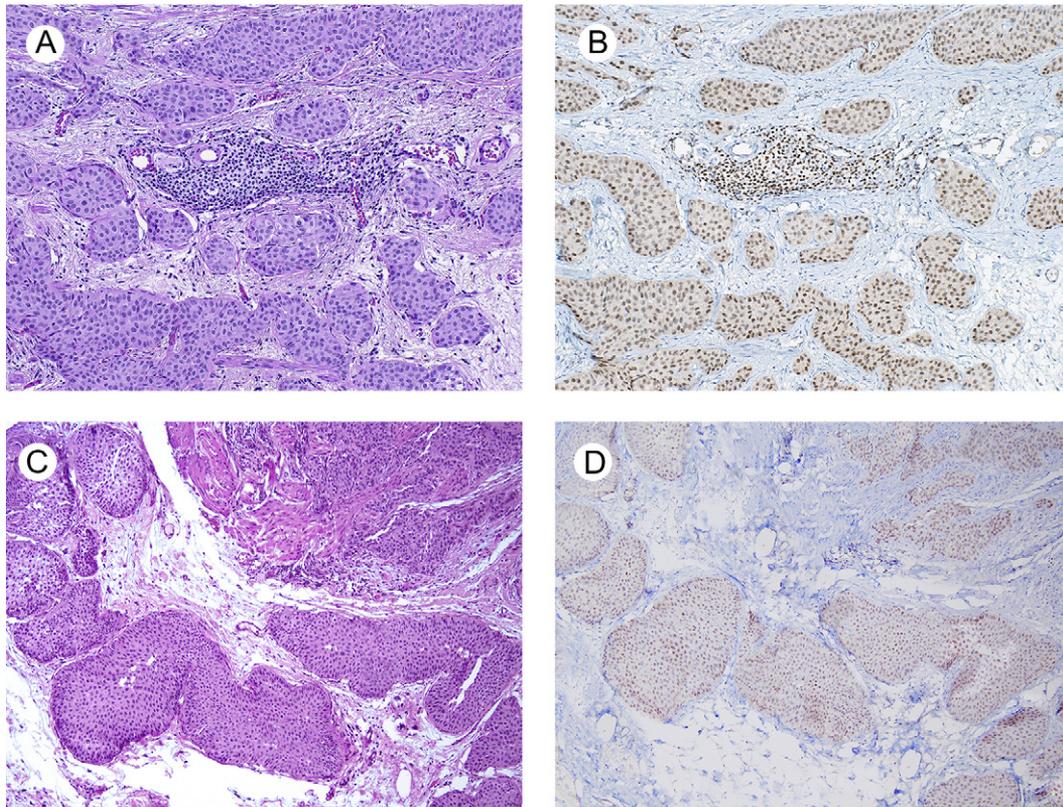


Fig. 1 A, Nested variant of urothelial carcinoma composed of solid nests. B, Same case as A with strong (3+) PAX8 expression. C, Urothelial carcinoma, large nested variant. D, Same case as C with moderate (2+) PAX8 expression.

nephrogenic adenoma, cystitis cystica, and cystitis glandularis. In particular, in small superficial tissue samples, nested variant of urothelial carcinoma with tubular formation can closely mimic nephrogenic adenoma. In challenging cases, immunohistochemical stains for PAX8 have been traditionally used, with a positive stain favoring nephrogenic adenoma. We have anecdotally seen expression of PAX8 in otherwise typical nested variant of urothelial carcinomas in the consultation service of one of the authors, and the purpose of this study is to formally explore the rate and extent of this expression and potential associated diagnostic pitfalls.

2. Materials and methods

2.1. Case selection

Electronic surgical pathology records were retrospectively searched for all cases diagnosed as nested variant of urothelial carcinoma at the Johns Hopkins Hospital from January 2011 to September 2018. Demographic data including age, sex, and anatomic location of the tumor were documented from patient records. Twenty (87%) of the cases were outside materials sent in for consultation to one of the authors and include biopsies and cystectomy

specimens. A representative block was selected, and hematoxylin and eosin (H&E) slides were prepared. The H&E slides were reviewed, and the architectural pattern of growth of the nested variant of urothelial carcinoma was documented. The patterns include solid nests, solid nests with tubules, and large nests.

2.2. Immunohistochemistry

Immunostaining was performed using a Bond max Leica autostainer (Leica Microsystems, Bannockburn, IL). Sections were deparaffinized, hydrated, and subjected to heat-induced antigen retrieval for 20 minutes with high-pH heat-induced epitope retrieval. Rabbit polyclonal anti-PAX8 (Protein Tech Group, Chicago, IL; 1:100 dilution) was applied for 15 minutes at room temperature followed by application of biotin-free bond polymer refine detection. The 3-3'-diaminobenzidine chromogen was used to visualize the reaction (all reagents from Leica Microsystems, Bannockburn, IL) and then counterstained with hematoxylin. The intensity of staining was classified as negative if there was faint (+1) or no staining, and positive if there was moderate (+2) or strong (+3) staining. Additionally, the extent of immunoreactivity was assessed as focal (positive in <50% of the cells) and diffuse (positive in \geq 50% of the cells).

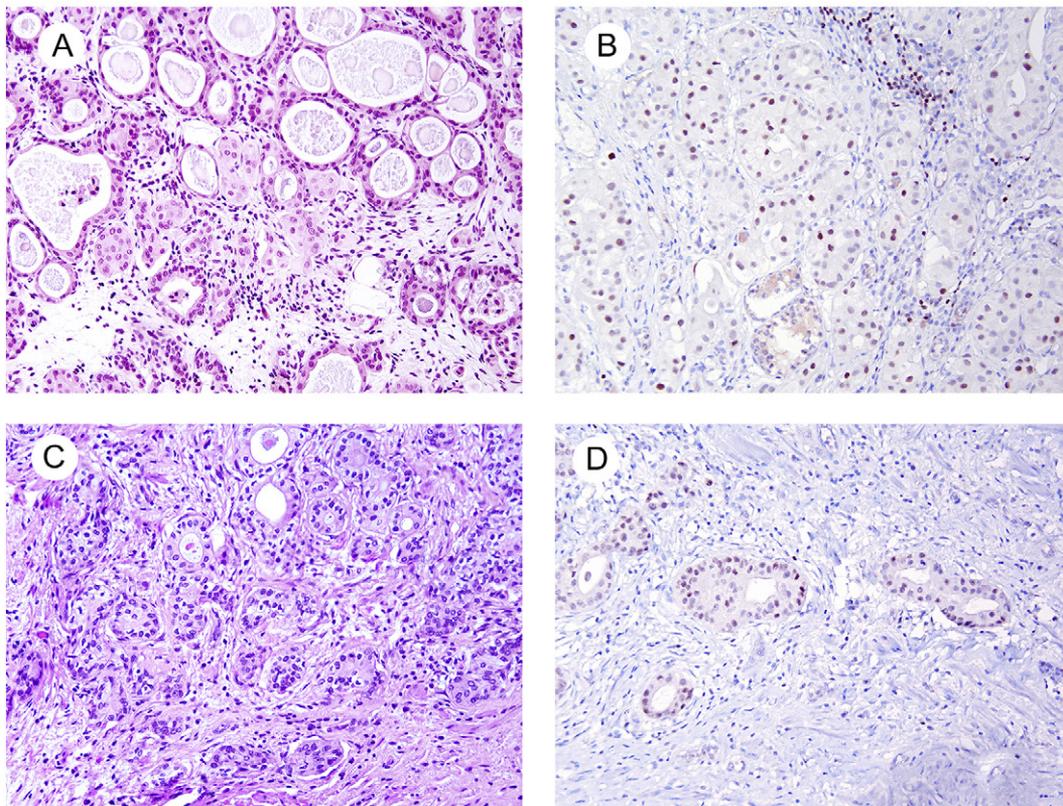


Fig. 2 A, Nested variant of urothelial carcinoma with predominantly tubular differentiation. B, Same case as A showing moderate (2+) PAX8 expression. C, Nested variant of urothelial carcinoma with solid nests and tubules. D, Same case as C with moderate (2+) PAX8 expression in the tubular component.

3. Results

A total of 29 cases were diagnosed as nested variant of urothelial carcinoma with tissue blocks or unstained slides available in 23 cases. The mean age at diagnosis was 63.7 years (range: 44-77 years), and the male to female ratio was 1.1:1 (Table). With the exception of 1 case, which was located in the renal pelvis, the remaining cases were from the bladder. All cases were from biopsies or transurethral resections, and none of the patients were treated with neoadjuvant chemotherapy. Six cases (26%) were composed of solid nests (Fig. 1A), with the majority (14/23; 61%) having solid nests with at least focal tubular differentiation (Fig. 2A and C). The remaining cases were of the large nested variant (Fig. 1C). PAX8 immunoreactivity was strong (3+) in 7 (30%) (Fig. 1B), moderate (2+) in 6 (26%) (Figs. 1D and 2B and D), and negative in 10 (44%) cases. Moderate-strong immunoreactivity was seen in 4/6 (66.7%) of cases with solid nests, 8/14 (57.1%) with solid nests and tubules, and 1/3 (33.3%) with large nests.

The only cases that had coexisting conventional urothelial carcinoma were 2 of the 3 large nested variants. One of these 2 cases showed strong PAX8 staining in 40% of the nests, and the overlying low-grade papillary urothelial carcinoma showed focal moderate PAX8 positivity. The other case with large nested urothelial carcinoma and low-grade papillary urothelial carcinoma was negative for PAX8 in both the components. None of our cases had urothelial carcinoma in situ or conventional invasive urothelial carcinoma.

4. Discussion

Nested variant of urothelial carcinoma is an aggressive tumor with outcomes similar to conventional urothelial carcinoma when compared stage for stage [4]. However, these tumors tend to be associated with a higher rate of locally advanced disease at diagnosis [4,5]. Wasco et al reported that, in comparison with pure high-grade urothelial carcinoma, nested variant of urothelial carcinoma was associated with muscle invasion at transurethral resection (31% versus 70%), extravesical disease at cystectomy (33% versus 83%), and metastatic disease (19% versus 67%) [5]. Similarly, Comp erat et al in their study of 36 large nested variant of urothelial carcinomas found that 58% of tumors at radical cystectomy had extravesical disease and/or lymph node metastases (\geq pT3 and/or \geq pN1) [6].

Architecturally nested variant of urothelial carcinoma is typically composed of closely packed, small, irregular nests with an infiltrative base. The lesions typically lack a prominent desmoplastic or inflammatory response. Confounding the diagnosis is that, typically, the urothelium overlying nested variant of urothelial carcinoma is normal without papillary or in situ carcinoma. Cox and Epstein also described a large

nested variant of urothelial carcinoma consisting of large, irregular invasive nests which are more commonly associated with a surface component [3]. The tumor cells in nested variant of urothelial carcinoma are generally medium-sized with abundant slightly granular cytoplasm with well-defined cell borders [7]. Cytological atypia tends to be focal and more commonly seen in deeper tissues. In addition to the low-grade cytology, additional features of malignancy such as mitotic activity, vascular invasion, and necrosis are not readily identified.

As a result, most of the morphologic differential diagnoses of nested variant of urothelial carcinoma are benign lesions such as von Brunn nests, cystitis cystica, cystitis glandularis, and nephrogenic adenoma. When nests of urothelium are noted in the muscularis propria, the diagnosis of nested variant of urothelial carcinoma is easier because von Brunn nests do not involve the muscularis propria. However, when the nests of urothelium are in the lamina propria, the distinction of nested variant of urothelial carcinoma and proliferation of von Brunn nests is more challenging. Even when there is muscularis propria involvement, if a predominantly tubular proliferation is present, the diagnosis can be difficult. The differential diagnosis includes nephrogenic adenoma, which can uncommonly involve the superficial muscularis propria, and nested variant of urothelial carcinoma with tubular differentiation. Especially in superficial and smaller tissue samples, the features can overlap and the distinction between these entities may be challenging. The importance of an accurate diagnosis cannot be overemphasized; misinterpretation of nested variant of urothelial carcinoma as benign can result in delay in treatment of an aggressive disease for which early diagnosis and treatment are the only option for a better outcome. On the other hand, misdiagnosis of benign lesions as malignancy can lead to overaggressive treatment with severe morbidity.

Prior studies have shown that nested variant of urothelial carcinoma have an immunohistochemical profile similar to conventional urothelial carcinoma. Wasco et al in a study of 30 cases of nested variant of urothelial carcinoma of the bladder demonstrated that they were positive for CK7 in 93%, CK20 in 68%, and p63 in 92% of cases [5]. Nested variant of urothelial carcinoma also shows expression of prognostic markers similar to those of poorly differentiated urothelial carcinoma: loss of p27 and higher p53 expression [8]. Additionally, Ki67 proliferation is generally higher than benign proliferative lesions such as von Brunn nests [8]. However, most of these markers show wide variation and overlap between nested variant of urothelial carcinoma and benign mimickers such that their use is limited.

As noted above, nested variant of urothelial carcinoma with tubular differentiation can closely mimic nephrogenic adenoma, particularly in smaller and superficial tissue samples [9]. To aid in this differential diagnosis, PAX8 immunohistochemical staining has been traditionally used, with a positive staining favoring the latter. PAX8 is a member of the paired box (PAX) gene family of transcription markers involved in the development of many organs [10]. It is a lineage-specific

marker expression which is limited to tissues of thyroid, Wolffian (nephric) ducts, and Müllerian ducts [11]. Whereas PAX8 is consistently expressed in nephrogenic adenomas, its expression in urothelial carcinomas is rare and mostly of upper tract origin. Although many studies report no staining for PAX8 in urothelial carcinomas, Laury et al in their comprehensive analysis of PAX8 expression in human epithelial tumors found that 3 of the 17 bladder urothelial carcinomas studied showed weak and/or focal staining. However, the subtypes of the urothelial carcinoma were not specified, and it is not clear if any of these cases were nested variant of urothelial carcinoma [12]. Albadine et al in their study of upper tract urothelial carcinomas found that 3 of their 34 cases showed PAX8 reactivity: 2 focal and 1 nonfocal in extent [13]. Tong et al found diffuse and moderate PAX8 staining in 23% of upper tract urothelial carcinomas but no expression in 40 bladder urothelial and 2 ureter urothelial carcinomas [14].

We found that a significant proportion of nested variant of urothelial carcinoma showed at least focal immunoreactivity to PAX8. The pattern of staining was similar in the different growth patterns of nested variant of urothelial carcinoma. A total of 21.7% of the cases had strong staining for PAX8 and 34.8% had moderate (at least focal) expression. It is not clear why a greater proportion of nested variant of urothelial carcinomas express PAX8 compared to conventional urothelial carcinoma. Prior studies have shown that the clinical features, biologic behavior, and molecular profiles of nested variant of urothelial carcinoma and conventional urothelial carcinomas are similar, suggesting that these lesions are related entities. For example, Zhong et al found similar rate of TERT promoter mutations between these 2 entities and in none of their 14 benign mimickers [15].

A potential weakness of the study is the relatively limited number of cases. However, nested variant of urothelial carcinoma is uncommon, and in many cases, blocks or unstained slides were not available for study from the outside institutions. Furthermore, our finding of PAX8 immunoreactivity in more than 50% of nested carcinomas would not change our conclusions if we have more cases. We did not include other benign mimickers such as von Brunn nests, as the major pitfall with PAX8 staining in nested carcinoma is with mimickers that are recognized to express PAX8 such as nephrogenic adenoma; if the differential diagnosis was between a proliferation of von Brunn nests and nested carcinoma, there is no reason why pathologists would do PAX 8 staining. This study focused on PAX 8 immunoreactivity in nested carcinoma because we had seen this unexpected staining create diagnostic difficulties in some cases sent to us in consultation. It was not the purpose of this study to examine PAX 8 staining across the spectrum of other variants of urothelial carcinoma which have greater cytological atypia and would not enter into the differential diagnosis with nephrogenic adenoma. PAX 2 staining was not

performed because it is less sensitive and has been replaced in practice with PAX 8.

In summary, we found that a significant proportion of nested variant of urothelial carcinomas express PAX8 at least focally. The expression is similar in the different histologic patterns of nested variant of urothelial carcinoma. This finding is important particularly in the differential diagnosis of nested variant of urothelial carcinoma with tubular differentiation versus nephrogenic adenoma. In challenging cases, one cannot rely on PAX8 immunoreactivity to differentiate between these 2 entities.

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