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Urinary Large Cell Neuroendocrine Carcinoma

A Clinicopathologic Analysis of 22 Cases

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Abstract: Large cell neuroendocrine carcinoma (LCNEC) of the urinary tract is a rare disease. We present a relatively large retrospective cohort of urinary LCNEC, 20 from the urinary bladder, and 2 from the ureter, from a single institution. The patients included 16 men and 6 women with a median age of 74.5 years. Most LCNEC presented at an advanced stage with tumors invading the muscularis propria and beyond (21/22). Eight cases were pure LCNEC, while 14 cases were mixed with other histologic types, including conventional urothelial carcinoma (n = 9), carcinoma in situ (n = 7), small cell carcinoma (n = 6), and urothelial carcinoma with glandular (n = 3) features. Most LCNEC expressed neuroendocrine markers synaptophysin (22/22), chromogranin (13/16), CD56 (7/7), TTF1 (8/8), and INSM1 (2/3). They were negative for common urothelial markers including HMWCK (0/3), p40/p63 (0/6), CK20 (0/10), and had variable GATA3 staining (4/8). Ki-67 stained 25% to nearly 100% tumor cell nuclei. Patient survival was associated with cancer stage, and pure LCNEC showed worse survival than mixed LCNEC. Compared with small cell carcinoma at similar stages from a prior study, LCNEC had a worse prognosis only when patients developed metastatic disease. For organ-confined LCNEC, neoadjuvant chemotherapy followed by radical resection is the treatment option to achieve long-term survival.

Key Words: large cell neuroendocrine carcinoma, urinary, bladder, clinicopathologic

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Bladder cancer is the sixth most common malignancy in the United States, with an estimated incidence of over 80,000 new cases and over 17,000 deaths in 2020.[1] Approximately 90% of bladder cancers are composed of urothelial carcinoma (UC), while other histologic types, such as squamous cell carcinoma and adenocarcinoma, are far less common.[2] Invasive UC demonstrates a high tendency to develop divergent differentiation, leading to a number of histologic variants, such as micropapillary, nested, plasmacytoid, and sarcomatoid.[3–5] Some aggressive UC variants are associated with poor clinical outcomes and may require therapeutic approaches that differ from those used for conventional UC.[6]

Primary neuroendocrine (NE) tumors of the urinary tract are rare, accounting for <1% of all urothelial neoplasms.[2,7,8] According to the 2016 World Health Organization Classification of Tumors of the Urinary System and Male Genital Organ, there are 4 subtypes of primary NE tumors of the urinary tract. They include small cell neuroendocrine carcinoma (SmCC) and large cell neuroendocrine carcinoma (LCNEC) (both high-grade and clinically aggressive), well-differentiated neuroendocrine tumor, and paraganglioma, which are more indolent.[2] LCNEC has only recently been recognized as a distinct entity in this classification. Due to the rarity of LCNEC, its biological and clinicopathologic characteristics remain largely unknown, which limits the development and evaluation of rational therapeutic strategies. Current knowledge of this disease is limited and is mainly based on small series and case reports.[9–28] Currently, there is no consensus on standard treatment for patients suffering from this aggressive malignancy. Herein, we describe the largest cohort of LCNEC, providing detailed clinicopathologic and immunohistochemical features of this rare disease.

MATERIALS AND METHODS

Patients

After obtaining the approval of the Institutional Review Board at the University of British Columbia (H18-03073), we searched our pathology database from 2006 to 2020. We found 22 cases of LCNEC of the urinary tract, including 20 from the urinary bladder and 2 from the ureter. All 22 patients underwent transurethral biopsy or

resection of the tumor, and 9 patients underwent radical cystectomy. The patients' archived hematoxylin and eosin–stained slides were retrieved from our archives and reviewed for pathologic analysis, including histologic features, other coexistent histologic variants, pathologic stage, and metastasis to lymph nodes and other organs.

Clinical data, including patient demographics, treatments, and outcomes, were retrieved from their medical records. The primary tumor pathologic stage was evaluated according to the 2017 American Joint Committee on Cancer TNM criteria.[29] In cases in which cystectomy was not performed, the clinical stage was determined based on clinical, transurethral biopsy or resection of the tumor, and radiographic findings.

Immunohistochemistry

Immunohistochemical staining was performed on routine sections. The following monoclonal antibodies were used for immunostaining: AE1/AE3 (clone AE1/3, 1:50 dilution; Dako, Carpinteria, CA), CK7 (clone OV-TL 12/30, prediluted; Dako), CK5/6 (D5/16B4 clone, prediluted; Dako), CK20 (clone Ks20.8, prediluted; Dako), synaptophysin (clone DAK-SYNAP, prediluted; Dako), chromogranin (clone LK2H10+PHE5, 1:200 dilution; Thermo Scientific, Waltham, MA), CD56 (clone 123C3, 1:50 dilution; Dako), TTF1 (clone SPT24, prediluted; Leica Biosystems, Buffalo Grove, IL), INSM1 (clone A-8, 1:150; Santa Cruz Biotechnology, Santa Cruz, CA), GATA3 (clone L50-823, 1:100 dilution; Cell Marque, Rocklin, CA), p63 (clone DAK-p63, prediluted; Dako), p40 (clone BC-28, 1:50 dilution; Biocare, Concord, CA), and uroplakin II (clone BC-21, 1:100 dilution; Biocare). Briefly, 4-μm-thick sections were deparaffinized in xylene and hydrated in graded alcohol. Immunostaining was performed using a DAKO autostainer (Agilent, Santa Clara, CA). Slides were incubated with the primary antibody and then with a visualization reagent (secondary goat anti-mouse immunoglobulin and horseradish peroxidase linked to a dextran polymer backbone). The slides were then rinsed with distilled water, incubated with a 3,3-diaminobenzidine substrate-chromogen solution, and subjected to Mayer hematoxylin counterstaining.

Statistical Analysis

The cancer-specific survival (CSS) for patients with LCNEC at different stages were compared using the Kaplan-Meier method with the SPSS program (IBM SPSS Statistics; IBM Corp., Armonk, NY). The CSS of urinary LCNEC were also compared with a cohort of SmCC from previous study.[30] The Fisher exact test was used to calculate 2-tailed *P*-values. *P*-values < 0.05 were considered statistically significant.

RESULTS

The patients included 16 men and 6 women (Table 1). The median age of patients was 74.5 years (range, 29 to 85 y). The most common initial presentation of the disease was gross or microscopic hematuria (n = 17). Other presenting symptoms included dysuria, increased urinary frequency, and urinary tract infection. Cystoscopy typically demonstrated ulcerated or fungating lesions. The tumors were most commonly found at the lateral wall (n = 9) and neck/trigone (n = 6), but occurred anywhere in the bladder. Tumor was found in a bladder diverticulum in 1 case and extended from the bladder neck into the prostatic urethra in another case. In 2 cases, the tumors were located in the ureter.

TABLE 1. Summary of Clinicopathologic Features of Urinary LCNEC

Features	No. Patients
Sex	
Male	16
Female	6
Tumor location	
Lateral wall	9
Posterior wall	2
Trigone/neck	6
Anterior wall	1
Dome	2
Ureter	2
Prostatic urethra	1
Diverticulum	1
Tumor histology	
Pure	8
Mixed with	14
UC	9
UC in situ	7
SmCC	6
Adenocarcinoma	3
Growth patterns	
Solid or cohesive sheets	14
Trabecular	8
Large nests	7
Special features	
Psuedorosettes	12
Peripheral palisading	5
Tumor necrosis	15
Primary tumor stage on cystectomy	
pT1	1
pT2	4
pT3	3
pT4	1
Clinical stage	
I	1
II	5
III	8
IV	8

Microscopically, the tumors cells were arranged in cohesive sheets (Fig. 1A), a solid growth pattern, large nests (Fig. 1B), or trabeculae (Fig. 1C). Peripheral palisading and rosettes were occasionally seen (Fig. 1D). The malignant cells were pleomorphic, of medium to large in size, round or polygonal in shape, with abundant light eosinophilic cytoplasm. The nuclei showed vesicular/fine chromatin and prominent nucleoli (Fig. 1E). There were numerous mitoses, apoptotic bodies (Fig. 1E), as well as tumor necrosis present (Fig. 1F). The morphology was similar to that of primary LCNEC of the lung.

Only 36% of LCNEC were pure (n = 8), and the majority were mixed with other histologic types (n = 14). Overall, the LCNEC component accounted for a mean of 53% (range: 25% to 90%) of the mixed tumor. The most

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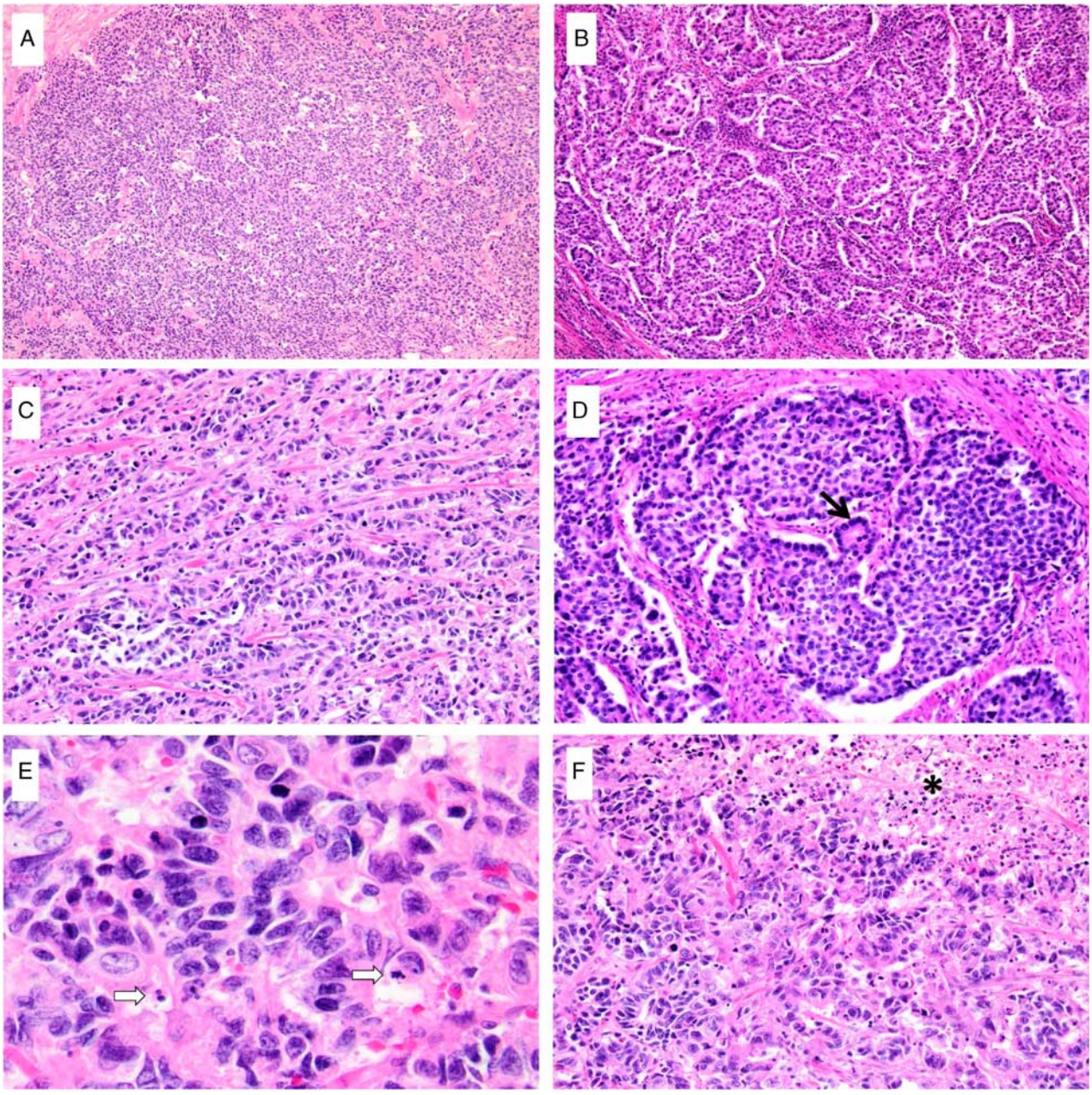

FIGURE 1. Histologic features of urinary LCNEC. A, Tumor with sheet-like growth pattern. B, Tumor with a large nest arrangement. C, Infiltrating tumor with a trabecular pattern. D, Peripheral palisading and rosettes (black arrow). E, High magnification showed malignant cells with pleomorphic medium to large cell size, round or polygonal shape with abundant light eosinophilic cytoplasm, vesicular/fine chromatin or frequent nucleoli, and apoptotic bodies (white arrows). F, Tumor necrosis (*).

common coexistent histologic type was conventional UC (n = 9) (Fig. 2A). Other histologic types included carcinoma in situ (CIS) (n = 7), SmCC (n = 6) (Fig. 2B), and adenocarcinoma (n = 3).

Immunohistochemical studies were performed in all cases (Table 2). All LCNEC expressed at least 1 NE marker, including synaptophysin (100%, 22/22) (Fig. 3A), chromogranin (81%, 13/16) (Fig. 3B), CD56 (100%, 7/7), TTF1 (100%, 8/8) (Fig. 3C), and INSM1 (66%, 2/3) (Fig. 3D). LCNEC also expressed various cytokeratins, such as pan-CK (100%, 13/13), and CK7 (75%, 9/12) (Fig. 3E), but the staining signals were often focal and weak. GATA3 staining was variable (4/8), ranging from completely negative (Fig. 3F), weakly/moderately positive (Fig. 3G), to diffusely strongly positive (Fig. 3H). Interestingly, all the pure LCNEC tested were negative for GATA3 (0/4). The tumors were negative for CK5/6 (0/4), p40/p63 (0/6), CK20 (0/10), and uroplakin II (0/1). Ki-67 stained 25% to nearly 100% of the tumor cell nuclei, in the 7 cases being examined.

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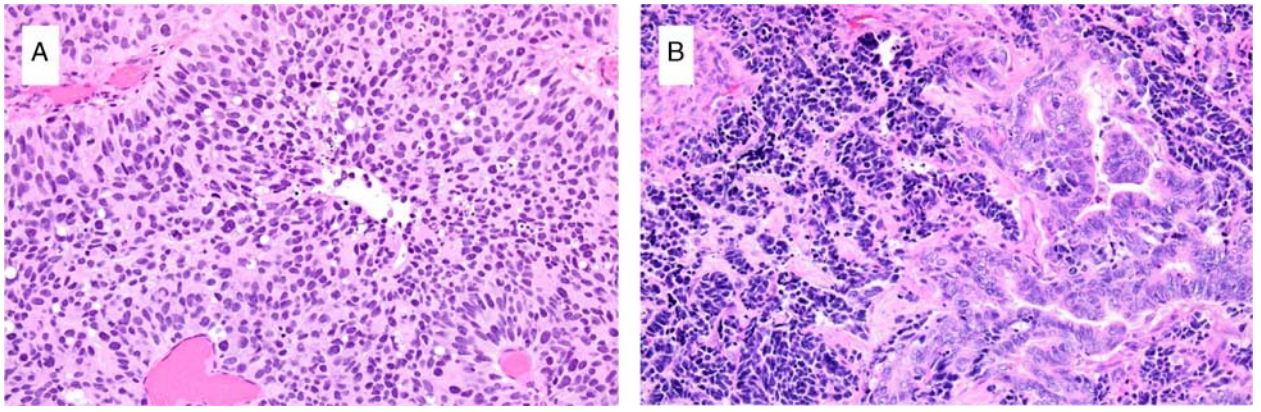

FIGURE 2. Urinary LCNEC coexisting with other histologic types. A, The most common coexistent histologic type was conventional UC. B, Coexistent SmCC (left) with LCNEC (right).

Disease staging was evaluated based on the pathology and imaging evaluations, including computed tomography, positron emission tomography, bone scan scintigraphy, and magnetic resonance imaging. At the time of diagnosis, 14 patients had diseases localized to the bladder/ureter, including stages I (n = 1), II (n = 5), and III (n = 8). Eight patients had stage IV disease with metastases to lymph nodes and other organs (n = 8). The most frequent sites of metastasis were liver (n = 5) and lung (n = 4), followed by brain (n = 2), bone (n = 2), adrenal gland (n = 2), pelvis (n = 2), retroperitoneum (n = 2), skin (n = 1), bowel (n = 1) and neck (n = 1).

Treatment information was available for all patients. Patients received chemotherapy (n = 12), radiation (n = 11), and cystectomy (n = 9). Among the patients who underwent cystectomy, 5 patients also received neoadjuvant chemotherapy before surgery.

Clinical follow-up was available for all patients, and the mean follow-up time was 23.5 months (range: 0.2 to 95.0 mo). Fifteen patients died within a mean of 13.0 months (range: 0.2 to 82.1 mo). One of these patients died from myocardial infarction and another patient died from angiosarcoma. Seven patients were alive after a mean of 46 months follow-up (range: 5.7 to 95.0 mo). The 1- and 5-year CSS rates were 47.0% and 33.6%, respectively, with a median CSS of 8.9 months. The median CSS for pure LCNEC was 3.5 months versus 40.5 months for mixed LCNEC, and the difference was statistically significant ($P = 0.03$) (Fig. 4A). The CSS was also significantly associated with the clinical stage ($P = 0.006$) (Fig. 4B). There was no significant difference in CSS between stage I to II and stage III, but CSS was worse in stage IV than those of stage I to II and III (Fig. 4B). In the 14 patients with no distant metastasis (stages I to III) who underwent neoadjuvant chemotherapy before cystectomy, the median survival time had not been reached at the median follow-up of 79.8 months. But the median survival time for patients at the same stage who did not receive neoadjuvant chemotherapy was only 8.9 months ($P = 0.05$, Fig. 4C). Long-term survival (> 60 mo) was observed in 4 patients who had disease localized to the bladder/ureter and received neoadjuvant chemotherapy.

The outcome of LCNEC was also compared with 77 cases of SmCC with clinical follow-up from a previous study.[30] LCNEC had a significantly worse median overall survival than that of SmCC (8.5 vs. 29.0 mo, $P = 0.026$), as well as worse CSS than SmCC (8.9 vs. 54.0 mo, $P = 0.039$, Fig. 4D). The CSS was not significantly different between LCNEC and SmCC in stage I to III patients ($P = 0.68$, Fig. 4E). However, LCNEC had a significantly shorter median survival time compared with SmCC for patients with distant metastasis (stage IV) (1.6 vs. 15.0 mo, $P = 0.02$, Fig. 4F). When the 6 mixed LCNEC/SmCC were removed from the LCNEC cohort, the survival analysis between LCNEC and SmCC showed a similar result as the above (Supplementary Figs. 1–3, Supplemental Digital Contents 1–3, http://links.lww.com/PAS/B153, http://links.lww.com/PAS/B154, http://links.lww.com/PAS/B155).

In the 8 stage IV LCNECs, 2 of them were mixed with SmCC. In the rest 6 stage IV LCNECs, 5 of them had multiple distant metastases, and one of them had

TABLE 2. Immunohistochemical Features of Urinary LCNEC

Antibody	Source	Dilution	No. Cases Tested	No. Positive Cases, n (%)
Synaptophysin	Dako	NA	22	22 (100)
Chromogranin A	Thermo	1:200	16	13 (81)
CD56	Dako	NA	7	7 (100)
TTF1	Leica	NA	8	8 (100)
INSM1	Santa Cruz	1:150	3	2 (67)
AE1/AE3	Dako	1:50	13	13 (100)
CK7	Dako	NA	12	9 (75)
CK20	Dako	NA	17	0 (0)
GATA3	Cell Margue	1:100	8	4 (50)
Uroplakin II	Biocare	1:100	1	0 (0)
p63	Dako	NA	3	0 (0)
p40	Biocare	1:50	4	0 (0)
CK5/6	Dako	NA	4	0 (0)

NA indicates not available.

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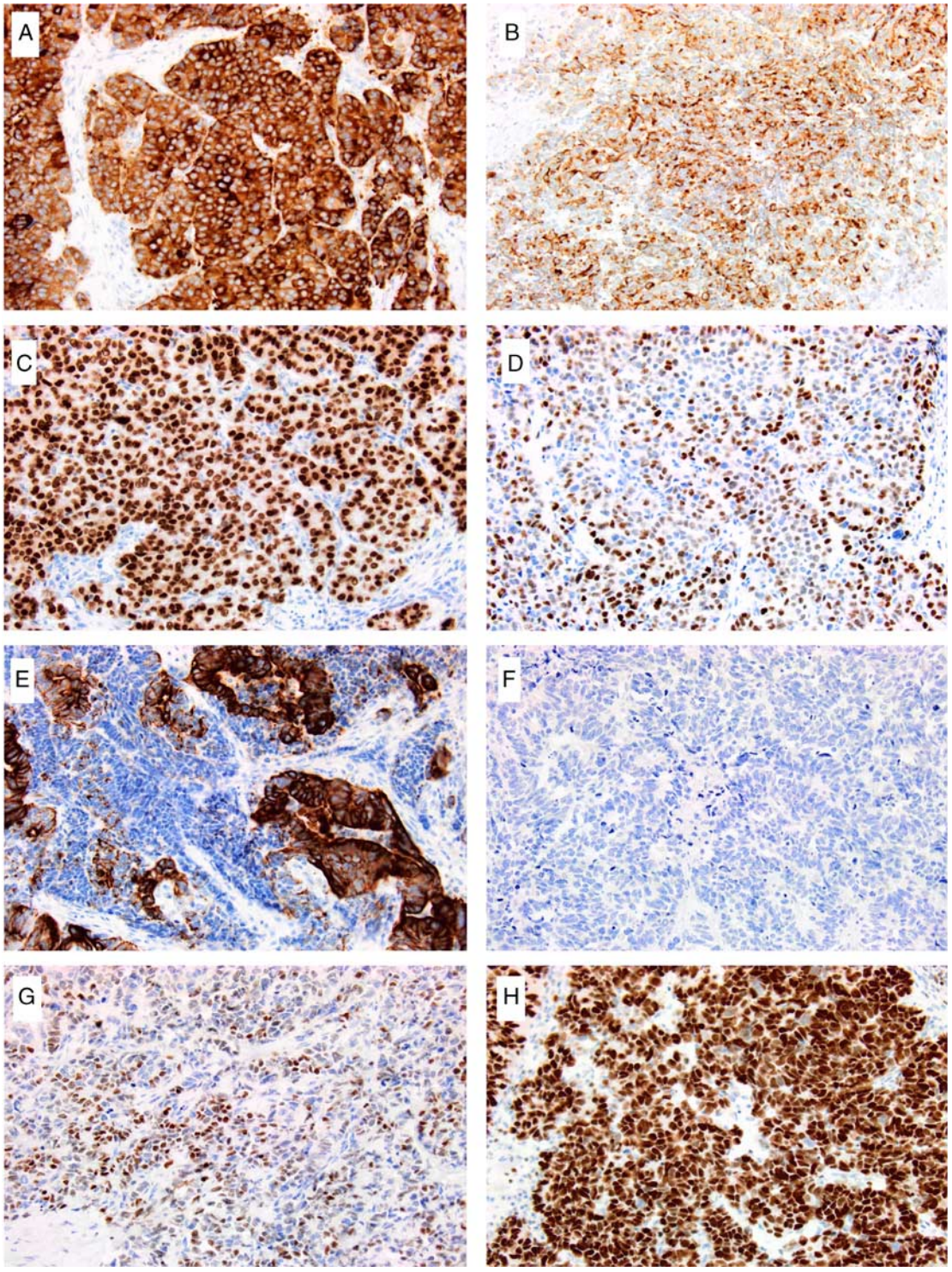

FIGURE 3. Immunophenotypical profile of urinary LCNEC. A, LCNEC was strongly positive for synaptophysin. B, LCNEC expresses chromogranin. C, LCNEC was strongly positive for TTF1. D, LCNEC was positive for INSM1. E, LCNEC was positive for CK7. Note the different staining patterns between LCNEC and adjacent SmCC. F–H, Variable staining percentage and density of GATA3 in LCNECs.

oligometastasis. In the 14 SmCCs, 9 of them had multiple distant metastases, and 5 of them had oligometastasis. The number of metastasis has no significant difference between them. We also collected more details of the therapy they received. For the 6 stage IV LCNEC patients, 3 of them with multiple metastases died soon after the diagnosis

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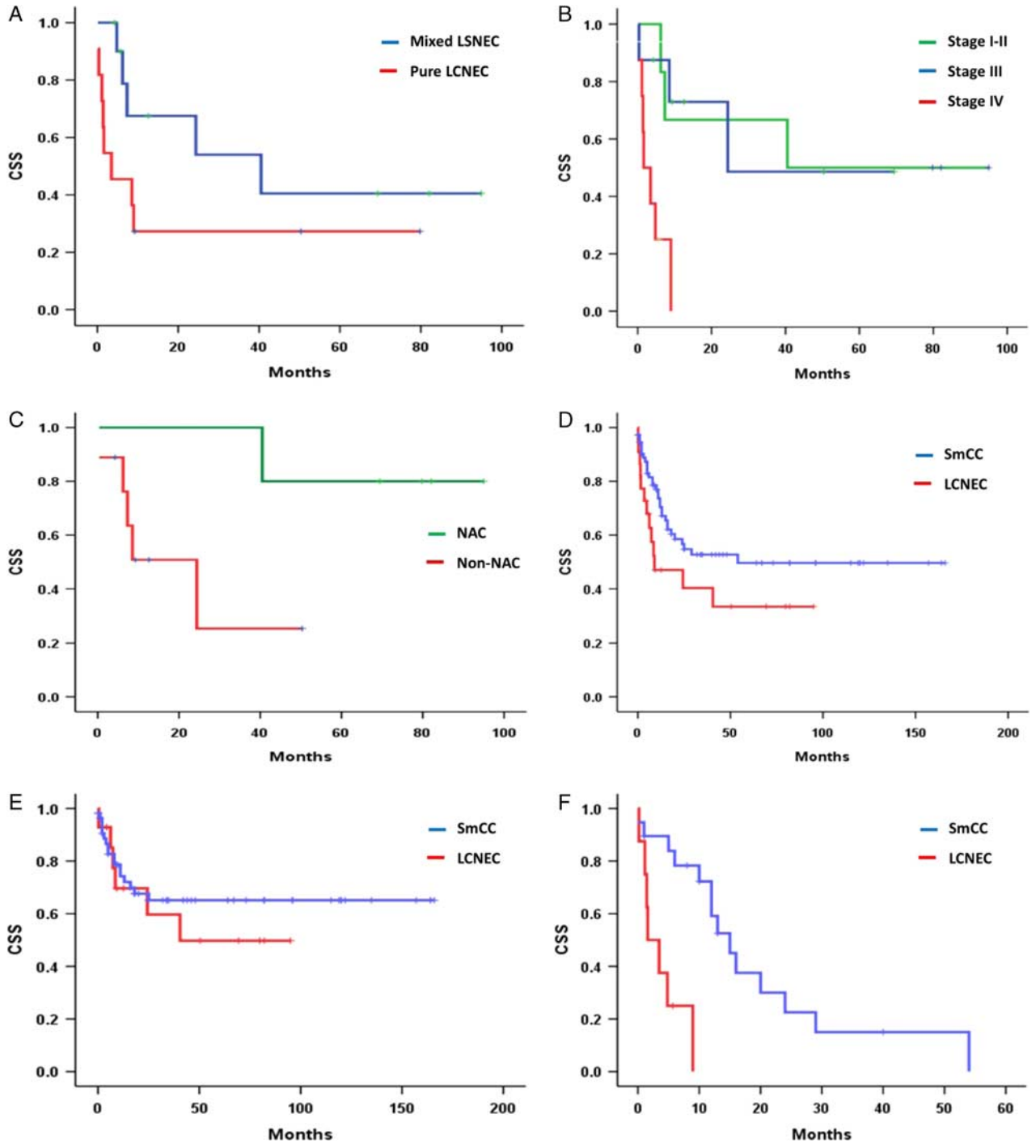

FIGURE 4. Kaplan-Meier survival analyses of urinary LCNEC. A, Pure LCNEC had poorer CSS than mixed LCNEC ($P = 0.03$). B, CSS is significantly associated with cancer stage ($P = 0.006$). C, Neoadjuvant chemotherapy (NAC) significantly prolongs survival ($P = 0.05$). D, LCNEC had poorer CSS than SmCC ($P = 0.039$). E, There is no significant difference in CSS between LCNEC and SmCC in stage I to III disease ($P = 0.68$). F, CSS for metastatic LCNEC is significantly worse than that for metastatic SmCC ($P = 0.02$).

(5, 34, and 43 postdiagnosis) before any therapy can be offered. Two other patients with multiple metastases received palliative radiation therapy, while the single patient with oligometastasis received chemotherapy. For the 14 stage IV SmCC, 4 of 5 patients with oligometastasis received neoadjuvant chemotherapy, radical cystectomy, followed by chemotherapy or radiation therapy, while the other received chemotherapy only. The SmCC patients with multiple metastases (N = 9) all received chemotherapy with or without radiation therapy.

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DISCUSSION

We retrospectively analyzed the largest cohort of urinary LCNEC so far from a single institution and found that LCNEC demonstrated clinicopathologic features distinct from conventional UC. At presentation, almost all (21/22) urinary LCNEC presented with invading the muscularis propria and beyond, and about 67% developed metastases to lymph nodes and other organs. CSS showed a significant association with the tumor stage. About 64% of urinary LCNEC were mixed with other histologic types, most commonly UC or UC in situ, and pure LCNEC showed a significantly worse survival than mixed LCNEC. Patients with LCNEC limited to the bladder/ureter achieved long-term survival after neoadjuvant chemotherapy and radical resection. We also compared the survival of urinary LCNEC and SmCC at similar stages, and our data suggested that, at stage IV, LCNEC had a significantly worse survival than SmCC, while the survival was comparable between them in stage I to III.

The overall prevalence of urinary LCNEC seems to be exceedingly low. Fewer than 50 sporadic cases have been reported in the literature so far, including both pure and mixed histology.[9–28] Nevertheless, since this tumor has been recently recognized as a distinct entity, it has been suggested that some urinary LCNECs might have gone underdiagnosed or just misdiagnosed as undifferentiated UC.[7] Similar to lung tumors, underdiagnosis and consequent underreporting remain an issue also in bladder cancer if appropriate diagnostic workup is not performed.[31] Urinary LCNEC usually affects elderly patients (above 60 y), although there is a wide age range at diagnosis (20 to 84 y, mean: 60.8 y),[32] consistent with our own findings. In addition, as previously reported,[33,34] LCNEC of the bladder had a male predominance (16/22) in our cohort.

Morphologic criteria for the diagnosis of bladder LCNEC are the same as its pulmonary counterparts.[35] Neoplastic cells are arranged in sheet-like, palisading, trabecular, or organoid nested growth patterns; single cells are large, polygonal, with abundant cytoplasm (usually light granular eosinophilic), and low nuclear to cytoplasmic ratio. Nuclei are polymorphic, often large, oval, featuring coarse, granular or vesicular chromatin, and often prominent nucleoli. Occasional bizarre cells may be seen.[20] Macroscopic or microscopic necrosis and/or frequent apoptotic bodies, as well as brisk mitotic activity (>10 mitoses/10 HPF) are usually seen. Rosettes or peudorosettes have been reported more often than in bladder SmCC.[16,17,23,35] Interestingly, as for the lung, bladder LCNEC displays high variability in cell size, and there is no clear nuclear or cell size cutoff between LCNEC and SmCC.[16,36] Therefore, some authors have suggested classifying all HGNECs as a single disease entity with case-to-case noting on its morphology to avoid the use of subjective criteria in distinguishing between small and large cells.[16,37] Nonetheless, our study demonstrated different morphologic features, overlapping but not identical immunostaining patterns, as well as different survival between LCNEC and SmCC, which should urge the pathologists to make the best effort to distinguish LCNEC from SmCC.

As a NE tumor, LCNEC expresses markers such as synapthophysin, chromogranin, CD56, and epithelial markers, namely pan cytokeratins, CAM 5.2, and EMA.[38] The above-mentioned immunohistochemical stains have a combined sensitivity and specificity of 96 and 100%, respectively, to distinguish from UC. According to the literature reports, CD56, synapthophysin, and chromogranin are expressed in most cases (100%, 92.6%, and 85.2%, respectively).[7] In our cohort, all LCNEC expressed at least 1 NE marker. LCNEC is a high proliferating tumor, with a Ki-67 index of up to 100%; however, a Ki-67 index of $>40\%$ has been shown to be 80% sensitive and 86% specific in distinguishing LCNEC and UC, which usually features a Ki-67 proliferation rate as high as 25%.[12,16,25,33] In the 7 cases in our cohort that were evaluated for Ki-67, 6 had a Ki-67 index of 75% or higher, with only 1 case having an index of 25%. Similar to previous studies, LCNEC also expressed various cytokeratins. It should be noted that LCNEC often shows diffuse cytoplasmic staining for CK and CK7, compared with the dots like staining pattern seen in SmCC. Furthermore, unlike SmCC, which is almost always negative for GATA3, LCNEC shows variable staining percentage and density for GATA3. We observed that the cases with positive GATA3 staining, especially those with strong staining, were the cases with more features of differentiation (peripheral palisading, pseudorosettes, and eosinophilic granular cytoplasm). However, since the sample size was small, we cannot conclude the results.

The differential diagnoses of urinary LCNECs include SmCC, poorly differentiated high-grade UC or prostate carcinoma, secondary involvement of the bladder by NE carcinoma from other sites, malignant lymphoma, malignant melanoma, paraganglioma/pheochromocytoma, lymphoepithelioma-like carcinoma, neuroblastoma, alveolar rhabdomyosarcoma, and metastatic Merkel cell carcinoma. Immunohistochemistry, and electron microscopy, if available, is useful in the distinction of LCNEC from non-NE lesions but is of limited value in differentiating lesions expressing NE markers, regardless of their histotype, grade, and organ of origin. Metastatic LCNEC needs to be ruled out, especially for pure LCNEC. Clinical history would be helpful to establish the diagnosis of primary urinary LCNEC. Therefore, it is essential to assess prior cancer history carefully. There are certainly some morphologic overlaps between LCNEC and high-grade poorly differentiated UC. Like its counterpart in the lung, LCNEC of the bladder usually demonstrate some NE features, including the architecture (organoid, nesting, palisading, trabecular, solid patterns, and rosette-like structures), and cytologic characteristics (large cells with abundant eosinophilic granular cytoplasm, variably coarse chromatin, nuclear pleomorphism, prominent nucleoli). As pathologists, when we see some of these features, we should raise the possibility of LCNEC and perform IHC to confirm it or rule it out. As demonstrated in the current study, LCNECs express NE

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markers, such as synaptophysin, chromogranin, INSM1, TTF1, and CD56. In contrast, UC usually expresses urothelial markers, such as uroplakin II and CK20. Unlike prostatic adenocarcinoma which often shows patchy/focal staining of NE markers, UC is generally negative for NE markers.[39] GATA3 would not be useful, since half (4/8) of LCNEC express GATA3, with density range from weak to diffusely strong. Metastatic LCNEC from other organs, particularly those from the prostate, may also involve the bladder. Urinary LCNEC frequently coexists with other malignant histologies, particularly UC and UC in situ. However, prostatic LCNEC often arises from and coexists with usual prostatic adenocarcinoma. Lymphoma and melanoma occasionally involve the bladder. In such cases, the patients' clinical history would often provide useful hints. Furthermore, lymphoma and melanoma express specific markers, so can be differentiated by immunostains.

Although the origin of urinary LCNEC remains uncertain, there have been several hypotheses about its pathogenesis. The most common hypothesis is that it originates from multipotent urothelial stem cells that can differentiate into various cell types. This is supported by the evidence of a heterogeneous immunophenotypical profile of urinary neuroendocrine carcinomas (NECs).[40] Another postulated theory is that urinary NECs may develop from malignant transformation of Kulchitsky-type NE cells in the bladder mucosa, as immunohistochemistry and electromicroscopy have demonstrated the presence of sporadic NE cells in the normal urothelium.[41] Other studies support the theory of transformation of UC cells. A recent study demonstrated that miR-145 could upregulate stem cell factors and induces cell senescence and differentiation into NE, glandular, squamous lineages.[42] In keeping with these findings, molecular genetic evidence, as well as the frequent coexistence of >1 histotype (namely, NECs, adenocarcinoma, or UC), suggests a common clonal origin.[43] In the current study and in the literature, approximately half of urinary LCNEC were mixed with other histologies, suggesting a close relationship between LCNEC and other UC subtypes.[3,12,14,15,23,36,37] Nonetheless, more genetic and molecular studies are needed to explore the oncogenesis of urinary LCNEC.

Pure LCNEC is associated with a worse prognosis compared with mixed forms in one study,[35] but not in 2 others.[33,37] This disparity may be due to the diverse sample sources (pooled case reports) or limited case numbers. In our study, which is the largest to date, pure LCNEC had a significantly worse prognosis than mixed tumors. We admit that the TUR might potentially miss some of the mixed components. In our cohort, 3/8 of "pure" LCNEC underwent radical resection, and none of them was found additional components in the radical specimen. 6/14 of "mixed" LCNEC underwent radical resection, and there was no significant difference between "pure" and "mixed" LCNEC for the procedure they received ($P=0.806$). Therefore, we speculate that the type of procedure would less likely impact the finding of mixed components, although we cannot rule out this possibility completely. The worse outcome of the pure LCNEC, compared with mixed, could be due to the disease stage at the time of diagnosis. In this cohort, 4/8 of pure LCNEC were diagnosed at clinical stage IV, while 4/14 mixed LCNEC were diagnosed at clinical stage IV, with no significant difference ($P=0.315$). But we are aware that the sample size in this study is small, and the significance might reach the cutoff of $P=0.05$ if the sample size is large enough.

Similar to SmCC, LCNEC is a biologically aggressive NE tumor, usually associated with a dismal prognosis despite an aggressive treatment.[9–28,34] Most clinical studies have analyzed both SmCCs and LCNECs, the latter comprising a small amount of the whole case series, and reported similar survival and cancer-specific mortality rates in LCNEC compared with SmCC, ranging from <1 year for advanced disease to >2 years for early disease.[7,12,15,16] Bhatt et al[10] reported a 5-year survival rate of 17% for their study population, which consisted of 14 SmCCs and only 4 LCNECs. Although our analysis is limited by comparison across study cohorts and our inability to conduct a multivariable analysis due to small sample size, it is the largest series of LCNEC in the literature and it suggests that the outcome of LCNEC may be worse than SmCC, especially for metastatic patients. We show some dependence of CSS on stage for LCNEC, which is consistent with previous studies in SmCC of the bladder.[30,44,45] Due to the small sample size and the diversity of the therapy strategies received, it is hard to conclude the relationship between the treatment and the survival in stage IV LCNEC versus SmCC. But it seems that the different survival between stage IV LCNEC and SmCC can be explained by the observation that half (3/6) of the stage IV LCNEC were diagnosed at very end stage and the disease progressed too quickly before any therapy can be received.

Due to its rarity, the optimal therapeutic strategy of the urinary LCNEC is debated, and no standard treatment exists. A single center study reported a difference in 5 patients treated with adjuvant chemotherapy versus surgery alone in terms of disease-free survival rates, but the finding was not conclusive due to the limited case number.[16] Chemotherapeutic regimens have been mostly extrapolated from those used for their pulmonary counterparts. Therefore neoadjuvant or adjuvant combination etoposide and platinum-based therapy is theoretically the treatment of choice.[10,33] Although surgery alone is not recommended in these cases, it plays a pivotal role in the optimal management of these patients. The limited evidence comparing surgery and bladder-sparing protocols is conflicting but suggests that radiation is less effective than radical surgery.[15,22] Due to the potential aggressive behavior of bladder LCNEC, prompt diagnosis and early treatment with neoadjuvant chemotherapy followed by radical cystectomy may provide long-term control of a localized tumor with extended overall survival,[9] as supported also by our data.

In conclusion, we reported so far the largest cohort of urinary LCNEC with detailed clinicopathologic and immunohistochemical analysis. LCNEC of the urinary tract is

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an aggressive disease that usually presents at an advanced stage with frequent metastases. Most urinary LCNEC are mixed with UC and other histologic types, suggesting that they might share a common clonal origin. Pure LCNEC showed significantly worse survival than mixed LCNEC. When the disease is localized, LCNEC does not have a significantly different clinical outcome from SmCC. However, when metastatic, LCNEC shows a significantly worse prognosis than SmCC. Neoadjuvant chemotherapy followed by radical resection can lead to long-term survival in patients who have localized LCNEC.

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