Primary MiNEN of the urinary bladder: an hitherto undescribed entity composed of large cell neuroendocrine carcinoma and adenocarcinoma with a distinct clinical behavior

Description of a case and review of the pertinent literature

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Received: 13 November 2020 / Revised: 28 December 2020 / Accepted: 6 January 2021
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Abstract
Neuroendocrine carcinomas (NECs) of the urinary bladder are very rare and can be observed in the context of mixed neuroendocrine/non-neuroendocrine neoplasms (MiNENs), most frequently in association with urothelial carcinoma. Small cell NECs are far more common than large cell NECs (LCNECs), which are exceedingly rare. We describe a primary MiNEN of the urinary bladder, composed of a LCNEC and of an adenocarcinoma, in which the neuroendocrine component reached complete pathological regression after neoadjuvant M-VAC chemotherapy, whereas the non-neuroendocrine component of the tumor progressed to metastatic disease. Compared to mixed neuroendocrine/non-neuroendocrine neoplasms described in the literature until now, this appears to be a unique case that expands the spectrum of neuroendocrine neoplasia of the urinary bladder.

Keywords Neuroendocrine neoplasm · Neuroendocrine carcinoma · Mixed neuroendocrine/non-neuroendocrine neoplasm · Urinary bladder

Introduction
Neuroendocrine neoplasms (NECs) of the urinary bladder represent less than 1% of all malignancies in this site and are mainly represented by Neuroendocrine carcinoma (NEC), whereas well-differentiated neuroendocrine tumors (NETs) are only anecdotally reported [1]. A significant proportion of NECs of the urinary bladder contains a non-neuroendocrine component, mostly represented by urothelial carcinoma and, more rarely, by squamous cell carcinoma or adenocarcinoma, and can be designated as mixed neuroendocrine/non-neuroendocrine neoplasms (MiNENs) in analogy to similar neoplasms arising in the digestive system [2]. Among vesical NECs, small cell NECs (SCNECs) are more frequently diagnosed than large cell NEC (LCNEC) [2, 3].

Here, we present a case of a MiNEN of the urinary bladder in which the neuroendocrine component, represented by a LCNEC, underwent complete pathological regression after neoadjuvant chemotherapy, while the non-neuroendocrine portion persisted and spread to metastatic sites.

Case history
A 49-year-old man was referred to the Urology Department for self-limiting painless gross hematuria in March 2018. Urinary cytology was positive for malignant epithelial neoplastic cells. Contrast-enhanced computerized tomography (CECT) showed a 46-mm-wide lesion
located on the dome of the bladder (Fig. 1). Transurethral resection of the bladder (TURB) was then performed, and the specimen was sent to the Pathology service. A diagnosis of MiNEN composed of LCNEC and adenocarcinoma of the bladder was signed out. Computed tomography of the brain, chest, and abdomen did not show metastatic disease. The patient received 3 cycles of neoadjuvant chemotherapy (methotrexate, vinblastine, adriamycin, and cisplatin—MVAC).

Radical cystoprostatectomy combined with the removal of pelvic and obturator lymph nodes was performed and a muscle-invasive poorly differentiated adenocarcinoma was reported, with no evidence of residual LCNEC. Three magnetic resonance imaging (MRI) scans of the abdomen were performed for clinical re-staging in January, May, and September 2019, respectively, without any evidence of relapse or metastatic disease.

In late November 2019, a growing lump on the penis and right epididymis was biopsied, revealing a poorly differentiated adenocarcinoma, without a neuroendocrine component. Emasculation was performed. After 2 years and 2 months after initial diagnosis, the patient is alive with ultrasonographic evidence of residual metastatic disease in inguinal lymph nodes.

**Materials and methods**

**Morphology and immunohistochemistry**

Tissue samples obtained from the different specimens (i.e., TURB, radical cystoprostatectomy, and percutaneous biopsy of the epididymis) were fixed in buffered formalin and routinely processed to paraffin wax. Five-micrometer-thick sections were routinely stained with hematoxylin and eosin and Alcian-PAS stain.

The immunohistochemical study was performed on additional 3-μm-thick sections using prediluted ready-to-use vials of the antibodies listed in Table 1 with an automated immunostainer (BenchMark Ultra, Ventana Roche Diagnostics) and standardized protocols (Ventana OptiView DAB IHC Detection Kit).

**Review of the literature**

The Pubmed database of the National Center for Biotechnology Information (NCBI) of the U.S. National Library of Medicine was searched using the keywords ‘MiNEN’ and ‘neuroendocrine carcinoma’. A total of 26 articles were identified for review. The results are summarized in Table 1.

| Antibody          | Manufacturer                  | Clone     |
|-------------------|-------------------------------|-----------|
| CD56              | Cell Marque Corporation*      | MRQ-42    |
| CDX2              | Ventana°                      | EPR2764Y  |
| Carcinoembryonic antigen (CEA) | Ventana°                   | CEA31     |
| Chromogranin      | Ventana°                      | LK2H10    |
| CK Cam5.2         | Ventana°                      | CAM5.2    |
| CK20              | Ventana°                      | SP33      |
| GATA3             | Cell Marque Corporation*      | L50-823   |
| Ki-67             | Ventana°                      | 30-9      |
| p16               | Ventana°                      | CINtec® p16 histology | |
| p53               | Ventana°                      | Confirm™ anti-p53 (DO-7) | |
| p63               | Ventana°                      | 4A4       |
| Rb1               | BD Biosciences®               | G3-245    |
| Synaptophysin     | Ventana°                      | SP11      |
| TTF1              | Ventana°                      | 8G7G3/1   |

*Cell Marque Corporation, Rocklin, CA, USA  
°Ventana Medical Systems Inc., Tucson, AZ, USA  
§ BD Biosciences, San Jose, CA, USA
Library of Medicine was searched using the following string “large cell neuroendocrine carcinoma [AND] urinary bladder.” All articles written in English were included. For each article, the reported cases were identified and, for each case, the following parameters were considered: age, sex, symptoms, presence of non-neuroendocrine component, immunophenotype, treatments, and outcome.

**Results**

**Morphology and immunohistochemistry**

The TURB specimen was entirely processed for microscopic analysis. Most of the specimens (70% of the total neoplastic volume) featured muscle-infiltrating neoplastic...
proliferation with organoid architecture, showing zonal necrosis (Fig. 2a). Neoplastic cells had moderately abundant, lightly eosinophilic cytoplasm, large vesicular nuclei, and focally prominent eosinophilic nucleoli. Apoptotic bodies were abundant and mitotic index was 40/10 high-power fields (HPFs) (Fig. 2b). Immunostains (Fig. 2c–h) were positive for Synaptophysin, Chromogranin A, CD56, CK Cam5.2, and, focally, for CK20 and TTF1. CDX2, GATA3, and p63 were negative. Intense cytoplasmic and nuclear p16 signal was also present, as well as p53 hyperexpression, whereas Rb1 expression was lacking. Ki67-related proliferative index was 85%.

The residual 30% of the total neoplastic volume was composed of an adenocarcinoma (Fig. 3), which was partially admixed with the former, but showed a tendency to be located in the most superficial layers of the bladder mucosa. Mitotic index was 4/10 HPFs. Immunostains for Synaptophysin, Chromogranin A, CD56, CEA, and p63 were negative, whereas those for CK Cam5.2, CK20, and GATA3 were diffusely positive and CDX2 was zonally expressed. Scattered cells were positive for TTF1. Rb1 was focally positive, while p16 and p53 had the same expression pattern as the neuroendocrine component. The final diagnosis was of muscle-invasive primary urinary bladder MiNEN, composed of LCNEC (70%) and moderately differentiated adenocarcinoma (30%).

The radical cystoprostatectomy specimen did not show, at gross evaluation, any residual neoplastic mass in the bladder. Microscopically, an estimated 90% of the vesical wall showed fibrosis and chronic inflammation with giantcell granulomas. In the remaining 10%, residual poorly differentiated adenocarcinoma was present, showing discohesive atypical cells with signet-ring-like and lipoblast-like features (Fig. 4). p63 and, focally, GATA3 were positive, but TTF1, CDX2, Chromogranin A, Synaptophysin, and Rb1 were absent. No residual LCNEC was identified.

In the percutaneous needle biopsy of the epididymis, poorly differentiated adenocarcinoma infiltrating fibromuscular tissue was seen (Fig. 5). Heterogenous positivity for GATA3 and p63 and negative stains for Chromogranin A, Synaptophysin, CD56, CD138, and PSA were observed. No evidence of LCNEC was found. The same morphological and IHC characteristics were observed in the specimen obtained from emasculation.
Review of the literature

We identified 25 articles published between 1986 and 2020, reporting a total of 41 cases of LCNEC of the urinary bladder (Table 2) [4–28]. The male-to-female ratio was 36:5 and patients’ age at diagnosis ranged from 20 to 84 years, with a median of 61 years. Specifically, 23 cases (56.1%) were pure LCNEC, 7 cases (17.1%) were a combined SCNEC/LCNEC [20, 23], 1 case (2.4%) had sarcomatous components [8], and 10 cases (24.4%) showed epithelial non-neuroendocrine components. Overall, the amount of the epithelial non-neuroendocrine components was small: in two cases, it was reported to account for less than 2% and less than 5%, respectively [6, 20]; in the remaining cases, a descriptive report was given (i.e., “evidence of,” “some foci of,” “minor contributions of” [16] epithelial non-neuroendocrine component).

Surgery and chemotherapy were the most frequently adopted treatments. Neoplasms were frequently muscle invasive, with or without fat infiltration, and commonly metastatic to regional lymph nodes. Outcomes were quite varied and based on follow-ups of different lengths.

Discussion

Our case is a rare example of what can be called a true MiNEN of the urinary bladder, as two morphologically distinct components, intimately admixed, one neuroendocrine and the
other non-neuroendocrine, were evident, both morphologically and immunohistochemically. In addition, this case is strictly adherent to the criteria used for digestive MiNENs [3], as each component represented at least 30% of tumor mass. In contrast, in previously reported cases of mixed vesical LCNECs, only a minor non-neuroendocrine component was detected [6, 9, 13, 16, 20]. Indeed, the adoption of a 30% cutoff is not based on clinical evidence, but rather it was arbitrarily introduced to avoid overestimating the biological relevance of focal cells with a divergent differentiation, which would be unlikely to influence the overall prognosis [29]. Nevertheless, as it has been underlined elsewhere [2, 29], we believe that minor, but morphologically recognizable, neoplastic components with divergent differentiation must be recorded in the pathological report, above all when they are morphological high-grade, because they still may influence prognosis and need a specific management.

LCNECs of the urinary bladder are exceptionally rare tumors, with only 41 cases reported in the literature (Table 1). Given their rarity, the exclusion of vesical metastatic disease from an unknown primary site is of paramount importance. Clinical and radiological information is pivotal in this task, as immunohistochemical markers have poor reliability in the identification of the primary sites of NECs [30]. In our case,
| Authors                  | Age/gender | Symptoms                          | Type                  | H&E                                      | Treatments                                      | Outcome                                      |
|-------------------------|------------|-----------------------------------|-----------------------|------------------------------------------|-------------------------------------------------|-----------------------------------------------|
| Lee et al. 2009 [16]    | 20, M      | Hematuria                         | Pure                  | CK33+, CK7+, NSE+, CD56+, CD68+, Chr+, Syn+, TTF1+ | Partial C, Ch, Rad                              | DOD 14 months after initial diagnosis        |
| Li et al. 2020 [17]     | 30, M      | Hematuria                         | Pure                  | CD56+, Chr+, Syn+, TTF1+                 | Partial C, Ch (cisplatin-etoxisplatin)          | AFD 22 months after surgery                  |
| Lee et al. 2006 [18]    | 35, M      | Hematuria                         | Pure                  | CK AE1/AE3+, NSE+, Chr+, Syn+, PSA+, LCA+, Vimentin+ | Partial C, Ch (M-VAC; gemcitabine, cisplatin) | AWD after 13 months; DOD after 13 months; DOD after initial diagnosis |
| Bertaccini et al. 2008  | 37, M      | Hematuria                         | Pure                  | CK7+, NSE+, Chr+, Syn+, TTF1+            | Partial C, Ch (cisplatin-etoxisplatin)          | AWD 22 months after surgery                  |
| Coelho et al. 2014 [4]  | 37, M      | Hematuria                         | Mixed                 | CK AE1/AE3+, NSE+, Chr+, Syn+, TTF1+     | Partial C, Ch (cisplatin-etoxisplatin)          | AFD more than 2 years after surgery Transferred to hospice about 13 months after surgery |
| Serrano et al. 2007 [20]| 40, F      | NS                                 | Pure                  | CK AE1/AE3+, NSE+, Chr+, Syn+, TTF1+     | Partial C, Ch (cisplatin-etoxisplatin)          | AWD 22 months after surgery                  |
| Akdeniz et al. 2018 [21]| 43, M      | NS                                 | Mixed                 | CK AE1/AE3+, NSE+, Chr+, Syn+, TTF1+     | Partial C, Ch (cisplatin-etoxisplatin)          | AWD 22 months after surgery                  |
| Colarossi, 2020 [31]    | 53, F      | NS                                 | Mixed                 | CK AE1/AE3+, NSE+, Chr+, Syn+, TTF1+     | Partial C, Ch (cisplatin-etoxisplatin)          | AWD 22 months after surgery                  |
| Abenoza et al. 1986 [24]| 55, M      | Hematuria and mucoid changes       | Mixed                 | CK AE1/AE3+, NSE+, Chr+, Syn+, TTF1+     | Partial C, Ch (cisplatin-etoxisplatin)          | AWD 22 months after surgery                  |
| Goret, 2020 [31]        | 70, M      | NS                                 | Pure                  | CK AE1/AE3+, NSE+, Chr+, Syn+, TTF1+     | Partial C, Ch (cisplatin-etoxisplatin)          | AWD 22 months after surgery                  |

**Table 2.** Published cases of large cell neuroendocrine carcinoma (LCNEC) of the urinary bladder

*H&E* = Hematoxylin and Eosin, *DOD* = Death of Disease, *TURB* = Transurethral Resection of Bladder, *nCh* = No Chemotherapy, *C* = Chemotherapy, *Ch* = Chemotherapy, *Rad* = Radiotherapy, *AWD* = Alive with Disease, *NS* = Not Specified.
Table 2 (continued)

| Authors            | Age/gender | Symptoms                  | Type              | IHCC*       | Treatments                                      | Outcome               |
|--------------------|------------|---------------------------|-------------------|-------------|------------------------------------------------|-----------------------|
| Chong et al. 2017  | 72, M      | Back pain, acute kidney injury | Pure CD56+, Chr+, Syn+ | C, nCh (carbo-eto), ADT | DOC 2 months after surgery | AWD 3 years after completion of treatments |
| Hailemarian et al. | 73, M      | Hematuria                 | Pure NSE+, Chr+, Syn+ | C           | Craniotomy, Ch (carbo-eto), whole-brain Rad | DOD 2 months after surgery |
| Tsugu et al. 2011  | 74, M      | Neurologic disturbances   | Pure CD56+, Chr+, Syn+, TTF1+ | Craniotomy, Ch, nCh, Doc, Partial C, Rad, whole-brain Rad | TURB 5 months after surgery |
| Evans et al. 2002  | 82, M      | Hematuria                 | Mixed CK A E1/AE3+, Chr-, Syn+, PSA-, Partial C, Rad, whole-brain Rad | AWD 2 years after initial diagnosis |
| Hata, Tasaki 2013  | 84, M      | NS                         | Mixed CD56+, Chr+, Syn+ | Mixed       | TURB 5 months after initial diagnosis |

Legend: *IHCC*: immunohistochemical; **ARF**: acute renal failure; **AFD**: alive, free of disease; **AWD**: alive without disease; **C**: cystectomy/cystoprostatectomy; **carbo-eto**: carboplatin-etoposide; **Ch**: chemotherapy; **Chr**: chromogranin; **CK**: cytokeratin; **Cr**: craniotomy; **Craniotomy**: craniotomy; **C**: chemotherapy; **DOD**: died of disease; **DOC**: died of other cause; **Dx**: diagnosis; **EMA**: epithelial membrane antigen; **E1/AE3**: epithelial membrane antigen; **F**: female; **HF**: heart failure; **HMWCK**: high-molecular-weight cytokeratin; **IHC**: immunohistochemistry; **LC**: lung cancer; **LCNEC**: large cell neuroendocrine carcinoma; **LCA**: leukocyte common antigen; **M**: male; **M-VAC**: methotrexate, vinblastine, doxorubicin, and cisplatin; **NA**: not available; **nCh**: neoadjuvant chemotherapy; **NS**: not specified; **NSE**: neuron-specific enolase; **PSA**: prostate-specific antigen; **PSAP**: prostatic-specific acid phosphatase; **Rad**: radiation therapy; **TURB**: transurethral resection of the bladder.
carcinomatous component, for which we endorse the term of MiNEN. The correct diagnosis on the preoperative biopsy allowed the administration of a platinum-based neoadjuvant polychemotherapy to the patient, which was followed by the complete pathological response of the LCNEC component, which did not recur in metastatic sites.

Authors’ contributions Giacomo Maria Pini: Conceptualization; investigation; and writing—original draft
Silvia Uccella: Conceptualization; investigation; methodology; project administration; resources; writing—review and editing; and supervision
Matteo Coriniti: Investigation; validation; and visualization
Maurizio Colecchia: Investigation; validation; and visualization
Giuseppe Pelosi: Investigation; validation; and visualization
Carlo Patriarca: Conceptualization; investigation; and supervision

Funding Open Access funding provided by Università degli Studi dell’Insubria.

Data availability All data generated or analyzed during this study are included in this published article

Compliance with ethical standards
Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study has been conducted according to the guidelines of the local ethical committee.

Consent to participate and for publication Informed consent has been obtained from the patient.

Code availability Not applicable

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