Analysis of Clinicopathological Features and Prognostic Factors in 39 Cases of Bladder Neuroendocrine Carcinoma

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Abstract. Aim: Through analysis and summarization of clinicopathological features, immunohistochemical expression, pathological diagnostic criteria, prognostic and other factors in patients suffering from bladder neuroendocrine carcinoma (BNEC), a better understanding of BNEC could be achieved to provide solid evidence for clinicopathology and prognosis. Materials and Methods: The clinicopathological data of 39 cases of BNEC with up to 5-year follow-up data (median follow-up=650 days) were analyzed retrospectively based on immunohistochemical staining. Survival analyses were carried out using the Kaplan–Meier method and tested with the log-rank method. Multivariate Cox regression analysis was adopted to screen independent risk factors affecting patients’ survival. In these 39 cases of BNEC, there were 26 cases of male patients, 13 female, with the proportion of male to female being 2:1. The ages of onset ranged from 44 to 86, with the median age being 62 and the average age 61.97 years, respectively. Histologically, referring to the WHO standard of neuroendocrine lung tumor classification, there were 7 cases of typical carcinoid tumors, 8 atypical carcinoid, 12 small-cell carcinomas and 12 large-cell carcinomas. In these cases there were 11 cases of featured urothelium carcinomas and 9 cases of adenocarcinomas. Results: Immunohistochemical staining showed that, in these 39 cases of BNEC, the positive expression for the neuroendocrine markers, including neural cell adhesion molecule 56 (CD56), synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), thyroid transcription factor-1 (TTF-1), cytookeratin (CK) and cytookeratin 7 (CK7), accounted for 39/39, 27/39, 18/39, 39/39, 19/39, 10/39 and 8/39, respectively. In contrast, cytookeratin 20 (CK20), protein 63 (P63), human melanoma black 45 (HMB45), S-lfln protein 100 (S-100) and leukocyte common antigen (LCA) were all negatively expressed. During the follow-up period, 12 patients died. The 1-, 3- and 5-year overall survival (OS) rates were 76.92%, 74.36% and 69.23%. Conclusion: BNEC is one of the most malignant tumors with severe invasiveness and poor prognosis. Immunohistochemistry revealed that CD56, Syn, CgA, NSE, TTF-1, CK, CK7, CK20, P63, HMB45, S-100 protein and LCA immune markers play important roles in diagnosis and differentiation. Many factors, including the patient’s age, size and shape of the tumor, operative method, perineuronal invasion, vascular invasion, distant organ metastasis and pathological type, show great difference in influencing OS time of patients, among which the size of the tumor, no invasion, vascular invasion and distant organ metastasis are independent risk factors affecting prognosis (survival time). Radical cystectomy is the prior alternative to treat this tumor.

Neuroendocrine tumors refer to the heterogeneous tumors stemming from peptidergic neurons and neuroendocrine cells in the diffuse neuroendocrine system, which may occur in organs and tissues in any part of the body (1). These tumors are likely to carry-out multi-potential differentiation secreting various active hormones, thus showing significant differences in biological behaviors and prognosis. They mostly appear in the lungs and digestive tract (2), rarely in the bladder. Thus, clinical, pathological and follow-up data were collected from 39 cases of bladder neuroendocrine carcinoma (BNEC) after diagnosis in the Department of Pathology between 2000 and 2015. Based on the data collected, understanding of BNEC can be deepened so that solid bases are set for clinical treatment and prognostic judgment.

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Materials and Methods

Materials. The 39 cases of BNEC originate from large specimens externally examined at Yantai Affiliated Hospital of Binzhou Medical University Hospital, the 5th People’s Hospital of Jinan, Yantai Yuhuangding Hospital and the Affiliated Hospital of Qingdao University from January 2000 to December 2015. All patients in these 39 cases had not undergone radiotherapy or chemotherapy prior to surgery. Postoperatively, 3 chief physicians jointly examined the radiographs under the microscope and drew their conclusions. Clinical data and summaries were collated from the medical record room and sample inspection sheet in the respective Hospitals, while the follow-up data were obtained by telephone interview or in the household registration Department of the local Public Security Bureau.

Methods. The samples from the 39 cases of BNEC were fixed with 10% neutral buffered formaldehyde, dehydrated and embedded in paraffin. The immunohistochemical streptavidin-peroxidase (SP) method was adopted, with primary antibodies being neural cell adhesion molecule 56 (CD56), synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), thyroid transcription factor-1 (TTF-1), cytokeratin 7 (CK7), CK20, human melanoma black 45 (HMB45), S-100 protein 100 (S-100), leukocyte common antigen (LCA) and 3,3'-diaminobenzidine (DAB) for staining. All reagents were purchased from Zhongshan Biotech Co. Ltd., Beijing, China. NSE, CK, Syn, CgA, CK7, CK20 and HMB45 stain positively in the cytoplasm and nucleus, LCA and CD56 stain positive in the membrane, whereas protein 63 (P63) stains positively in the nucleus.

Follow-up. In this study, all 39 cases of BNEC were followed up by phone and with the household registration Department of the local Public Security Bureau. Another 28 cases were excluded due to lack of available follow-up data. Follow-up data enabled us to discern the survival time and the patients’ outcomes. The duration of the research started from the confirmation of diagnosis by the respective Pathology Department to the death of the patients or the end of the study.

Statistical analysis. Data analysis was performed using statistical software SPSS17.0. (http://www.spss.com) The enumeration data were compared with the $χ^2$ test. Survival analyses were analyzed using the Kaplan-Meier method and tested with the log-rank method. Multivariate Cox regression analysis was adopted to screen independent risk factors affecting the survival of patients, with differences being statistically significant when $p<0.05$.

Results

Clinical data. In these 39 cases of BNEC, 26 cases were males and 13 females, with the proportion of male to female ratio being 2:1. The age of onset ranged from 44 to 86 years with a median age of 62 and average age of 61.97, respectively. In 24 cases, patients were over the age of 60 years. There was no statistical significance between the males’ and the females’ age of onset ($p=0.819$). Nineteen of the 39 cases showed tumors on the left lateral wall, 12 on right lateral wall, 2 at right parietal area, 4 on right posterior wall and 2 in the bladder neck. Ultrasonics, in most cases, revealed a thickened bladder wall, which was the site of the occupying lesion (Figure 1A). The clinical symptoms of 25 cases featured hematuria in part, if not the whole course of the study. In these 25 cases, 15 patients described pain and/or discomfort in the waist and/or abdomen. Ten cases showed urinary irritation symptoms, whilst 4 cases had no significant symptoms upon physical examination. The course of illness varied from 1 day to 2 years. Only in 12 cases did the patients smoke. Of the 26 cases that were subjected to radical total cystectomy treatment, 5 cases had partial cystectomy and 8 cases transurethral resection of the bladder tumor.

Pathological features. Nine of the 39 cases of BNEC showed the shape of a mushroom, 1 polypoida, 26 ulcer and 3 lobular. The diameters of tumors ranged from 0.8 cm to 13 cm, with the median diameter being 3 cm and average diameter 3.42 cm, respectively. There were 13 cases of vessel invasion, 10 cases with no invasion and 11 cases of distant organ metastasis. Referring to the WHO standard of neuroendocrine tumor classification (3), there were 7 cases of typical carcinoid (Figure 1B), 8 cases of atypical carcinoid (Figure 1C), 12 cases of large-cell carcinoma (Figure 1D), 1 case of small-cell carcinoma (Figure 1E), 3 cases of urothelial carcinoma (Figure 1F) and 9 cases of mixed tumors (Figure 1G).

![Figure 1](image-url)
Figure 2. Continued
1C), 12 cases of small-cell carcinoma (Figure 1D) and 12 cases of large-cell carcinoma (Figure 1E). Among them, 11 cases featured urothelium carcinoma (Figure 1F) and 9 cases adenocarcinoma (Figure 1G). According to the tumor-node-metastasis (TNM) classification of bladder cancer, there were 15 cases of phase I, 4 cases of phase II, 2 cases of phase III and 18 cases of phase IV cancer.

**Immunohistochemical staining.** The positive expression of 39 cases of BNEC for the neuroendocrine markers included CD56 (Figure 1H), Syn (Figure 1I), CgA (Figure 1J), NSE (Figure 1K), TTF1 (Figure 1L), CK (Figure 1M) and CK7 (Figure 1N) were 39/39, 27/39, 18/39, 39/39, 19/39, 10/39 and 8/39, respectively. In contrast, CK20, P63, HMB45, S-100 protein and LCA were all negative.

**The relationship between clinicopathological features and prognosis.** In this study, only 39 cases had follow-up data from the day of diagnosis to December 31st, 2015. The follow-up lasted from 60 days to 1,800 days, with median follow-up lasting 650 days. In this period, 12 patients died with survival time averaging 290.58 days. The 1-, 3- and 5-year overall survival (OS) rates were 76.92%, 74.36% and 69.23%, respectively.

**The effect of gender on survival rate.** In the 39 cases of BNEC, there were 26 male patients (66.67%) 10 (38.46%) of whom died. In the 13 female patients (33.33%), 2 (15.38%) died. Differences in survival rates between different genders were not statistically significant over the whole survival period (p=0.227) (Figure 2A).

**The effect of age on survival rate.** In the 39 cases of BNEC, there were 15 (38.46%) cases in which patients were younger than 60 years of age, 4 (26.67%) of which died during the course of this study. There were 24 (61.44%) cases with patients over the age of 60 years, 8 (33.33%) of which died. Differences in survival rate between these two age groups was considered statistically significant in the OS period (p=0.013) (Figure 2B).

**The effect of the sizes of the tumors on survival rate.** In the 39 cases of BNEC, there were 25 (64.10%) cases in which tumor size was ≤4 cm. Of these 25 patients, 4 (16%) died. Tumors larger than 4 cm appeared in 14 (35.90%) cases, of which 8 (57.14%) patients died. Differences in survival rate between these two tumor size groups was considered statistically significant in the OS period (p=0.001) (Figure 2C).

**The effect of the shape of the tumor on survival rate.** In the 39 cases of BNEC, 26 (66.67%) cases showed the shape of an ulcer, of which 6 patients died. Of the 9 cases with mushroom-like tumors, 3 patients (33.33%) died. There was 1 (2.56%) case of polypoida in which the patient survived the follow-up period. Three (7.69%) cases of the 39 featured a lobular shape; of these patients, all 3 (100%) died. Survival rate differences between the differently shaped tumors was considered statistically significant over the course of the follow-up period (p=0.001) (Figure 2D).
The effect of smoking on survival rate. In the 39 cases of BNEC, 12 (30.77%) patients were smokers, of which 4 (33.33%) died. Twenty seven patients (69.23%) had no history of smoking, of which 8 (29.63%) died. The differences in survival rates between these two groups did not reach statistical significance during the follow-up period (p=0.867) (Figure 2E).

The effect of operative methods on survival rate. In the 39 cases of BNEC, 8 (20.51%) cases underwent transurethral resection of their bladder tumors, of which 2 (25%) patients died. There were 5 (12.82%) patients who underwent partial cystectomy, of whom 1 (20%) died. Twenty six (66.67%) patients underwent radical total cystectomy, with 9 (34.62%) dying. Differences in survival rates among transurethral resection of bladder tumors, partial cystectomy and radical total cystectomy were considered statistically significant during the follow-up period (p=0.026) (Figure 2F).

The effect of vessel invasion on survival rate. In the 39 cases of BNEC, 13 (33.33%) cases had vascular invasion, of which 7 (53.85%) patients died. There were 26 (66.67%) cases in which there was no evidence of vascular invasion, with 9 (34.62%) patients dying. Differences in survival rates between these two groups, of vascular invasion did not reach statistical significance during the follow-up period (p=0.001) (Figure 2G).

The effect of no invasion on survival rate. In the 39 cases of BNEC, 10 (25.64%) cases had no invasion; of these 10 patients, 4 (40%) died. No sign of perineuronal invasion appeared in 29 cases (74.36%); however, 8 patients (27.59%) died. Differences in survival rates among transurethral resection of bladder tumors, partial cystectomy and radical total cystectomy were considered statistically significant during the follow-up period (p=0.016) (Figure 2H).

The effect of distant organ metastasis on survival rate. In the 39 cases of BNEC, 11 (28.20%) cases had distant organ metastasis, with 8 (72.23%) patients dying. There were 28 (71.79%) cases in which there was no sign of distant organ metastasis but, nevertheless, 4 (14.29%) patients died. Differences in survival rates between these two groups were statistically significant over the total follow-up period (p=0.001) (Figure 2I).

The effect of pathological types on survival rate. In the 39 cases of BNEC, there were 7 (17.95%) cases of typical carcinoid with no deaths noted in the follow-up period. Eight (20.51%) patients had a typical carcinoid; 2 (25%) died. Of the 12 (30.77%) cases of small-cell carcinoma, 6 (50%) patients died. There were 12 (30.77%) cases of large-cell carcinoma where 4 patients (33.33%) died. Differences in survival rates among typical carcinoid, atypical carcinoid small-cell carcinoma and large-cell carcinoma were statistically significant (p=0.027) (Figure 2J).

Results of prognostic multivariate analyses. Multivariable Cox regression analysis selecting principal variables revealed that the size of the tumor, perineuronal invasion, vascular invasion and distant organ metastasis are independent risk factors affecting the prognosis of the BNEC patients (survival time). Factors are shown in Table I.

Discussion

Epidemic investigations show that NEC in bladder is quite rare, only accounting for 0.5-1.0% (3, 4) of all malignant tumors in the bladder, with no specificity (5, 6) in clinical manifestation and imaging features. Up to now, there is no confirmed reason for BNEC. It has been reported both domestically and abroad that BNEC is closely related to smoking (7-9). However, in relation to the 12 smokers included in our study, no statistical differences in survival rates between smokers and non-smokers were seen during the 5-year follow-up period. In order to solve this puzzle, we need to enlarge the scale of sampling to further investigate the role of smoking in BNEC. The most vulnerable group of people for BNEC tends to be older men, with the main clinical manifestation being painless gross hematuria. Some patients also had symptoms of dysuria, frequency and urgency of micturition and urodynia. BNEC mostly appears on the lateral walls, followed by posterior and back walls. Under cystoscope, tumors had mushroom, polypoida, ulcer or lobular shape (6). Concerning gender, age, clinical symptoms and tumor location, there were 26 male and 13 female cases, with those over 60 accounting for 24 out of the 39 cases (61.54%). The clinical symptoms of 25 cases featured gross hematuria in part, if not the whole, of the

Table I. Multivariable Cox regression analysis of overall survival.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4 cm</td>
<td>1.01</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt;4 cm</td>
<td>(1.47-6.93)</td>
<td></td>
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<tr>
<td>Vessel invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5.92</td>
<td>0.002</td>
</tr>
<tr>
<td>No</td>
<td>(1.57-22.3)</td>
<td></td>
</tr>
<tr>
<td>Never invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5.47</td>
<td>0.034</td>
</tr>
<tr>
<td>No</td>
<td>(1.35-22.0)</td>
<td></td>
</tr>
<tr>
<td>Distant organ metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26.60</td>
<td>0.001</td>
</tr>
<tr>
<td>No</td>
<td>(5.98-118.2)</td>
<td></td>
</tr>
</tbody>
</table>

HR, Hazard ratio; CI, confidence interval.
should be considered along with comprehensive analyses in
Firstly, high-grade urothelium carcinoma has obvious
a lightly-stained cytoplasm and immunohistochemical
staining shows specificity of tumor cells expressing CK7 with
no expression of neuroendocrine markers, such as CD56, Syn
and CgA. The second to be identified is lymphoma. The nucleolus is large and clear with mitotic count,
which is easily seen. Immunohistochemistry of tumor cells
reveals S-100 protein staining. In our group of cases, S-100 was
negatively expressed, which was helpful in differentiation. The seventh disease, without obvious differences in
tumor tissue displays, in the nest type, rich interstitial blood
vessels. Tumor cells are round or polygonal of the same size
with few Sertoli cells around the nest. Although immuno-
histochemistry of tumor cells revealed presence of Syn and
gA, differentiation could be ascertained by the negative
staining of CK-pan and EMA. Sertoli cells feature positive S-
100 protein staining. In our group of cases, S-100 was
negatively expressed, which was helpful in differentiation.
Fifthly, lymphoepithelioma-like carcinoma (LELC) needs to be mentioned. Tumor cells show flaky or nest-like shapes of
different sizes. The size of tumor cells is very large, with
bubbly nuclei. The nucleolus is very clear with abundant
lymphocytes and plasma cells. In this work, immunohistochemical staining of tumor cells showed only
epithelial markers, without expression of neuroendocrine
markers, which can be used for identification. Sixthly,
metastatic small-cell carcinoma is another topic to be
discussed. There are clinical records showing malignant
tumors in other body parts. When urothelium carcinoma
features appear histologically, it helps the diagnosis of the
primary tumor. What is noticeable is that BNEC can also
express TTF-1, as is shown in 19 of the 39 cases presented
in this report. Therefore, this marker cannot be used for the
differentiation of primary and metastatic tumors. The seventh
disease that needs to be distinguished from BNEC is prostatic
small-cell carcinoma invading the bladder. Three cases of
small-cell carcinoma of the urinary bladder invading prostate
are included in this work, which are very similar to this
seventh disease, without obvious differences in
immunophenotyping. Therefore, it is hard to differentiate
these two distinct cases. We consider that the pathogenic sites
course. In these 25 cases, patients in 15 cases suffered from
pain in the waist or (and) abdomen. Ten cases showed
urinary irritation symptoms, while 4 cases had no significant
symptoms upon physical examination. Nineteen out of the
39 cases showed tumors on the left lateral wall, 12 on right
lateral wall, 2 at right parietal area, 4 on right posterior wall
and 2 in bladder neck. Nine of the 39 cases of BNEC
showed mushroom, 1 polypoid, 26 ulcer and 3 lobular
shape. All of these equate with reports in the literature (9).

Diagnosis of BNEC mainly depends on histopathology,
immunohistochemistry and ultrastructural features, similar to
NEC in the lung and other tissues (10-12). NEC in large cells
of the bladder is fairly rare, with only 85 case-reports up to
now. In these 85 cases, 35 simple and 50 mixed-type cases
have been reported. Little is known about the clinical data
of BNEC. In our study, 10 of the 12 cases had mixed
features with urothelium carcinoma and adenocarcinoma.
Immunohistochemistry staining convincingly suggests that
diagnosis of BNEC, with other frequently used immuno-
histochemical markers, should be performed with the use of
Syn, CgA, CD56 and NSE markers. Alijo et al. (11) have
reported 44 cases of small-cell carcinoma of the urinary
bladder and 2 cases of large-cell neuroendocrine carcinoma
(LCNEC). Of them, small-cell carcinoma positively
expressed Syn (50%), CgA (30%), CD56 (68%), NSE (80%)
and TTF-1 (100%). As shown in our data, the 12 cases of
small-cell carcinoma positively expressed CD56 (12/12), Syn
(11/13), CgA (8/12), CK17 and TTF-1 (9/13) to different
degrees. This result revealed that CD56, Syn and CgA show
greater positive rates than previously stated in the literature.
In our 12 reported cases of LCNEC, 3 neuroendocrine
markers, including Syn, CgA and CD56, were all positively
expressed (12/12). This is different from the literature
mentioned above (11) and may be attributed to the clonal
code of the antibodies used. Further studies are required with
a larger scope of sampling. TTF-1 is a specific marker of
thyroid and pulmonary tumors. According to our data, 19 out
of 24 cases of the BNEC positively expressed TTF-1,
including 11 cases of small-cell carcinoma and 8 cases of
large-cell carcinoma. These markers cannot be used as an
index of identification for metastatic small-cell carcinoma in
the lung. Instead, clinical data and other immune markers
should be considered along with comprehensive analyses in
the laboratory.

In view of the special prognostic significance, it is
important to identify the following diseases in diagnosis.
Firstly, high-grade urothelium carcinoma has obvious
pleomorphic cells of large size and rich cytoplasm. They have
a lightly-stained cytoplasm and immunohistochemical
staining shows specificity of tumor cells expressing CK7 with
no expression of neuroendocrine markers, such as CD56, Syn
and CgA. The second to be identified is lymphoma. Malignant lymphoma, which is primarily present in bladder,
organ metastasis are independent risk factors affecting the prognosis of the patient (survival time). Without other reports available, either domestic or international, further studies with enlarged sampling should be carried out to exclude the effect of limited sampling and ensure the reliability of this research. For treatment, most scholars suggest combining radical bladder dissection and chemotherapy to improve clinical effectiveness. Of course, there are also some scholars adopting neoadjuvant chemotherapy, which has been shown to have good efficacy (4). In recent years, the development of molecular targeted therapy for malignant tumors has advanced and some investigators suggest that molecular targeted therapy of BNEC using epidermal growth factor receptor (EGFR), B-cell lymphoma 2 (Bel-2) and CD56 might hold promise, potentially bringing significant improvement to the survival rate of BNEC patients (17).

References


