

Answering to Reviewer #1

1. Comment: The study's introduction is fairly simple and should be rephrased into three paragraphs that communicate the following in order: - What is the importance of this study? The first paragraph of the study's introduction must provide a response to this question. - The second paragraph should describe the knowledge gap that the present research seeks to address. - The third paragraph should explain the present research challenge and propose a strategy for resolving it within the constraints of the current study's purpose.

Answering: Thank you very much for your suggestions. We found them very useful and tried to revise the Introduction section according to them. We divided the introduction into three paragraphs. The first paragraph states that our case will provide valuable data for other researchers, and our tortuous diagnosis process and less commonly used treatments can provide a reference for clinicians. The second paragraph describes the current cognitive status of LCNEC of the bladder. The disease is progressing rapidly, but there is no standard treatment, and the treatment for urothelial cancer is not suitable for LCNEC. The third paragraph describes the similarities between this disease and urothelial carcinoma of the bladder, and failure to distinguish between the two can lead to inappropriate treatment. We suggest that attention should be paid to distinguishing this rare tumor from urothelial cancer before surgical treatment.

2. Comment: Some of the references to the present study are outdated and have no value in the context of the current research. I recommend revising the sources to include

references from 2024 and five years before that.

Answering: Thanks for your suggestion, it made us realize that references 7-9 are outdated. In the revised manuscript we have replaced them with new, more informative references.

Answering to Reviewer #2

1. Comment: Although the article mentions potential cellular origins of LCNEC, it lacks a deeper discussion on the pathogenic mechanism and associated risk factors. Understanding the molecular and cellular context could be as crucial as the clinical case itself.

Answering: We will be happy to edit the text further, based on helpful comments from the reviewers. We reviewed the latest literature and made a new discussion on the LCNEC of bladder from the aspect of gene mutation, which was added to paragraph 1 of the Discussion section of the manuscript. However, the bladder is a very rare site for extrapulmonary LCNEC. Unfortunately, we have not found more conclusive evidence to verify its pathogenesis.

2. Comment: The manuscript could have benefited from a more extensive comparison with other reported cases, as well as a discussion of how the treatment of this case aligns with or differs from established or emerging protocols for LCNEC.

Answering: We added a new discussion about the comparison of other cases in paragraph 5 of the Discussion section of the manuscript. We reviewed the treatment and follow-up results of other cases of LCNEC of the bladder reported in the literature. We

compared the effects of different treatment schemes as a basis for our choice of partial cystectomy.

3. Comment: While the treatment decision based on patient conditions and preferences is mentioned, a more profound discussion of the available treatment options and their evidential bases would strengthen the manuscript.

Answering: We added a new discussion about the comparison of other cases in paragraph 5 of the Discussion section of the manuscript. We increased the discussion on the prognosis of different surgical procedures. We found that the effect of partial cystectomy was similar to that of radical cystectomy, and partial cystectomy had less harmful to the quality of life of patients. This can be used as a basis for recommending partial cystectomy.

4. Comment: The manuscript underscores the relevance of early diagnosis and comprehensive treatment, yet it does not offer clear recommendations or guidelines for physicians who may encounter similar cases.

Answering: Thank you so much for the advice, it's very valuable. We have added specific recommendations to improve diagnostic accuracy before surgery in paragraph 4 of the Discussion section of the manuscript. For patients with a definite diagnosis of LCNEC of the bladder, we provide specific treatment recommendations in paragraph 5 of the Discussion section. These recommendations include the rationale for choosing partial cystectomy.

5. Comment: It would be interesting to append a more detailed discussion on the

diagnostic challenges and strategies to improve the preoperative diagnostic accuracy of LCNEC.

Answering: I believe this suggestion will make our manuscript more valuable. We have added a more detailed discussion of the problems in the diagnosis of LCNEC of the bladder in the revised manuscript. In addition, for the diagnostic challenges, we put forward specific recommendations to identify LCNEC of the bladder. This section is added to paragraph 4 of the discussion section of the manuscript.

Answering to Science Editor:

1. Comment: The language classification is Grade B and Grade B.

Answering: Before submitting the revised manuscript, we corrected the grammar of our manuscript through the professional English language editing companies and uploaded the Non-Native Speakers of English Editing Certificate.

2. Comment: Manuscript Title

Answering: We have revised the title of the manuscript to "Primary large cell neuroendocrine carcinoma of the bladder: A case report" as required by the journal.

3. Comment: Author contributions does not meet the requirements.

Answering: We have revised the statement of the author's contribution describing the specific contribution(s) made by each author.

4. Comment: References.

Answering: Before submitting the revised manuscript, we have used "Edit References by

Auto-Analyser" to edit the references of the manuscript.

5. Comment: Figures.

Answering: We modified the abbreviations of the legends in figures 2 and 3 according to the "Guidelines for Authors".

6. Comment: The manuscript must ensure a clean page and no annotations or line numbers are allowed.

Answering: Before submitting the revised manuscript, we have removed line numbers.

7. Comment: Informed consent forms need to be processed to protect patient privacy.

Answering: We re-uploaded the revised Signed Consent for Treatment Form, which protects the patient's privacy.

Other: Our first draft format refers to "Format_for_Manuscript_Submission-Case_Report" provided by the "Guidelines for Authors", which contains the "FURTHER DIAGNOSTIC WORK-UP" section. When we submitted the revised version, we found that this title was not on the page, so we merged this part into the "TREATMENT" section.

Name of Journal: World Journal of Clinical Cases

Manuscript Type: CASE REPORT

Primary large cell neuroendocrine carcinoma of the bladder: A case report

Bai LL et al. LCNEC of the bladder

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Author contributions: Bai LL designed the study and wrote the manuscript. Jiang YQ checked and audited the paper. Guo YX was in charge of patient treatment. Song SY and Li R prepared figures. All authors gave final approval for the version to be submitted.

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Abstract

BACKGROUND

Large cell neuroendocrine carcinoma (LCNEC) is a rare non-urothelial tumor of the bladder. The treatment of LCNEC of the bladder is different from that of urothelial carcinoma (UC); therefore, early and accurate diagnosis is particularly important. As LCNEC of the bladder is rare and its clinical symptoms and radiographic features are similar to those of urothelial tumors, the clinical diagnosis of the disease remains challenging.

CASE SUMMARY

We report a 72-year-old female patient who presented with gross hematuria for 3 months. A solitary tumor located in the anterior wall of the bladder was found by

cystoscopy. Pathological examination after biopsy suggested UC of the bladder in the absence of immunohistochemical assessment. The patient underwent partial cystectomy and was finally diagnosed with LCNEC (pT2bN0M0) based on the results of postoperative immunohistochemical examination. During the ten-month follow-up, we found no signs of tumor recurrence or metastasis.

CONCLUSION

Immunohistochemical examination is essential for diagnosing LCNEC of the bladder. Accurate diagnosis and multidisciplinary treatment in the early stage of the disease are crucial for improving the prognosis.

Key Words: Large cell neuroendocrine carcinoma; Bladder tumor; Pathology; Immunohistochemistry; Partial cystectomy; Case report

Core Tip: Large cell neuroendocrine carcinoma (LCNEC) of the bladder is a rare non-urothelial tumor of the bladder. As LCNEC is a rare disease and its clinical symptoms and radiographic features mimic those of urothelial tumors, it may be misdiagnosed as urothelial carcinoma. We reported a patient with LCNEC of the bladder, who was not accurately diagnosed due to the absence of immunohistochemical examination before surgery. In this article, we summarize the methods for diagnosing and treating LCNEC of the bladder and highlight the importance of early diagnosis and multimodal treatment.

INTRODUCTION

Neuroendocrine carcinoma (NEC) is commonly found in the lung and gastrointestinal tract but rarely found in the urinary system [1]. NEC of the bladder accounts for less than 1% of bladder tumors [2], and LCNEC is the rarest subtype [3,4]. Few cases of LCNEC of the bladder have been reported previously, and studies on its biological and clinicopathological features are lacking. We report a patient with LCNEC of the bladder, who was not accurately diagnosed due to the absence of

immunohistochemical examination before surgery. The treatment process and clinical data can improve clinicians' awareness of LCNEC of the bladder and facilitate its accurate diagnosis and treatment.

LCNEC of the bladder has a poor prognosis, with the majority of patients being diagnosed at an advanced stage [5,6]. However, due to the rarity of the disease, there is currently no standard treatment. The treatment of UC of the bladder is not applicable to this disease. In the existing studies, the most commonly used treatment was comprehensive treatment including surgery [2,5]. The therapeutic effect of new antineoplastic drugs, such as targeted drugs and immune checkpoint inhibitors, on this disease is still uncertain [5,7,8]. More data are needed to develop guidelines for the diagnosis and treatment of this disease.

As the clinical symptoms and radiographic features of LCNEC of the bladder are similar to those of urothelial tumors, it may be misdiagnosed as UC; thus, clinicians cannot choose the appropriate treatment in a timely manner. The diagnosis of this disease is challenging for clinicians. This article summarizes the diagnostic and treatment approaches for LCNEC of the bladder. We suggest that attention should be paid to the identification of rare pathological types of bladder tumors before surgery to adopt timely and appropriate treatment.

CASE PRESENTATION

Chief complaints

A 72-year-old female patient who had experienced intermittent gross hematuria in the last 3 months came to our hospital.

History of present illness

The patient experienced gross hematuria 3 months before her visit, with obvious terminal hematuria accompanied by a burning sensation in the lower abdomen, without frequency or urgency. After treatment with oral cefixime for 1 wk, there was no change in hematuria; therefore, she came to the Department of Urology at the

Hebei Medical University Third Hospital for treatment. Ultrasonography revealed a hypoechoic mass in the anterior wall of the bladder.

History of past illness

The patient had no history of chronic diseases or other diseases.

Personal and family history

The patient was exposed to leather tanning reagents for more than 10 years. The patient did not mention any family history.

Physical examination

Physical examination showed no obvious positive signs.

Laboratory examinations

Urine analysis showed that RBC count was 418.40/ul and WBC count was 46.50/ul.

Imaging examinations

Contrast-enhanced CT revealed that the tumor involved almost the whole layer of the bladder wall and exhibited mild enhancement, similar to what is observed in common infiltrative UC of the bladder (Figure 1A, B).

FINAL DIAGNOSIS

LCNEC of the bladder (pT2bN0M0).

TREATMENT

After admission, cystoscopy revealed a pink mass with a maximum diameter of approximately 2 cm on the anterior wall of the bladder (Figure 2A). A small amount of tumor tissue was obtained by cystoscopy and sent for pathological examination. The pathological section showed pronounced atypia, large and differently shaped

cells and nuclei, and deeply stained nuclei (Figure 2B). Tumor cells invaded the muscularis layer. The pathologist diagnosed a malignant tumor of the bladder, suggesting an invasive UC. The patient was unwilling to pay for immunohistochemical examination; thus, the pathologists could not determine the origin of the tumor. The preliminary clinical diagnosis was bladder UC (cT2N0M0). As the patient wished to retain her bladder, we opted for laparoscopic partial cystectomy rather than radical cystectomy. During the operation, no tumor invasion or adhesions were found in the adipose tissue outside the bladder wall. Pathological examination of the complete tumor showed that the tumor invaded the deep muscular layer rather than the vessels or nerves, and no tumor tissue was found at the incisal margin or base. The morphology of the cells revealed large nuclei, coarse-grained chromatin, a nucleolus, mitosis, and trabecular growth patterns (Figure 3A). Immunohistochemical examination revealed that the cells were positive for chromogranin-A (CgA) and synaptophysin (Syn) (Figure 3B, C) and negative for CD56. The Ki-67 index reached 70% (Figure 3D). The patient was finally diagnosed with LCNEC of the bladder (pT2bN0M0) based on the results of immunohistochemical examination.

OUTCOME AND FOLLOW-UP

The patient refused further chemotherapy after the surgery. During the ten-month follow-up, we detected no indications of tumor recurrence or metastasis.

DISCUSSION

The tissue origin of LCNEC of the bladder is still unclear. Recent studies have suggested that the metaplasia of neuroendocrine cells, pluripotent stem cells, or urothelial cells beneath the bladder mucosa are possible origins [9]. Since nearly half of all LCNECs in the bladder contain tumor cells from other tissue types, such as UC, small cell carcinoma, or adenocarcinoma [2,10], pluripotent stem cells have become popular because of their origin. Recent studies have identified mutations of multiple genes, such as P53, RB1, NTRK, RET, and BRAF, in LCNEC of the bladder.

Inactivation of TP53 and RB1 is the most common genetic mutation in the LCNEC of the bladder, but unfortunately, neither of these genes can be a target for precision therapy [11]. Future studies on gene targets may bring new treatment options for patients with LCNEC of the bladder.

The average age at diagnosis of LCNEC of the bladder is 61.5 years, and the male-to-female ratio is approximately 4:1 [2]. Smoking and personal or family history of cancer are risk factors for this cancer [4], but our patient did not mention any of these factors. However, whether her exposure to leather tanning chemicals contributed to her disease development remains unclear. Hematuria is the most common clinical symptom of LCNEC of the bladder [2,10]. At the time of diagnosis, most patients have muscle layer invasion or distant metastasis [2,10], which may be related to its high invasiveness and lack of early symptoms.

CT and MRI can help the diagnosis and staging of LCNEC of the bladder. Some studies have recommended 18F-FDG-PET/CT scanning to measure the systemic change in 18F-FDG uptake since tumor cells in NEC have high metabolic activity and high 18F-FDG uptake [3,5]. As a functional imaging modality, 18F-FDG-PET/CT is more reliable than CT or MRI for staging the disease [11]. This advanced method is not available in most hospitals and cannot be fully applied.

Currently, the diagnosis of LCNEC relies on histopathological examination and immunohistochemical staining. The pathomorphological characteristics of LCNEC of the bladder include large cells with pleomorphism, abundant cytoplasm, coarse granular chromatin, and prominent nucleoli. In addition, LCNEC of the bladder sometimes shows special structures, such as rosettes, palisading pattern, and trabecular architecture [8,10,11]. The most important method for diagnosing NEC is immunohistochemical examination. The expression of Syn, CgA, and CD56 and a Ki-67 index > 40% indicate high sensitivity and specificity for detecting NEC compared to UC [5,8-10]. In the present case, palisade-like structures, large nuclei, coarse granular chromatin, and nucleoli were observed in the surgically resected tumor tissue (Figure 3A), and immunohistochemical staining showed positive Syn and CgA and a Ki-67 index of 70% all supporting the diagnosis of LCNEC (Figure 3B-D), even

though CD56 was negative. For this patient, the pathological sections of the tissue obtained during cystoscopy showed the general characteristics of malignant tumor cells, including large cells and nuclei, deep staining of nuclei, and lack of cell polarity. Rosette and palisade-like structures were not observed possibly because the tissue amount was too small and could not represent most of the diseased tissue. It is difficult to make an accurate diagnosis based on limited histological and morphological information. Immunohistochemical examination serves as a reliable method to distinguish NEC from other tumors. Cystoscopy and urine cytology are common methods to obtain pathological specimens. Urine cytology has a relatively high false negative rate, even though it is a non-invasive test. In comparison, cystoscopy is a more reliable method. However, in certain scenarios, such as when imaging examination confirms the presence of bladder tumors, cystoscopy may be bypassed. Although UC is the most common type of bladder tumor, the differential diagnosis of rare tumors should not be overlooked. Patients with bladder tumors confirmed by imaging examination should undergo cystoscopic biopsy to accurately determine the origin and stage of the tumor. Accurate diagnosis is crucial, as non-urinary epithelial tumors necessitate different treatment modalities compared to urothelial cancer. Currently, there is no standard treatment for LCNEC of the bladder. The common clinical treatments include surgery, chemotherapy, and radiotherapy. Compared to surgery alone, multimodal treatment is more conducive to prolonging survival [4,10,12]. There was no significant difference in overall survival between patients who underwent radical cystectomy and patients who underwent bladder-sparing surgery [2]. The same chemotherapy regimens used for small cell neuroendocrine carcinomas of the lung, such as etoposide and platinum drugs, are adopted for treating LCNEC of the bladder. Cisplatin combined with etoposide or carboplatin combined with etoposide is the treatment choice [9]. A study showed that cisplatin-based chemotherapy was superior to carboplatin in terms of prolonging the survival time [13]. For our patient, preoperative imaging revealed no extravesicular invasion or metastasis. Compared to radical surgery, bladder-sparing surgery may lead to similar survival times but has less impact on the quality of life. Our patient refused

postoperative chemotherapy or radiotherapy. Single-modal treatment may be a risk factor for poor prognosis. We conducted a review of the follow-up data of patients with bladder LCNEC, comparing the prognosis of various surgical procedures. We observed that similar to those undergoing partial cystectomy, 33% of patients treated with radical cystectomy suffered from metastasis or recurrence during follow-up. Patients treated with trans-urethral resection of the bladder tumor had the worst prognosis, with 50% having metastasis or recurrence during follow-up [5]. We recommend radical surgery combined with platinum chemotherapy for patients with LCNEC of the bladder. For localized tumors that can be completely removed from the bladder, partial cystectomy may be an alternative option, particularly for patients refusing radical surgery. However, currently, few patients undergo partial cystectomy whose reliability needs more studies. Our patient had a relapse-free survival of 10 months after partial cystectomy, without any additional treatments. She had a favorable prognosis, showing the value of partial cystectomy. We will continue to closely monitor her condition.

The overall prognosis of LCNEC of the bladder is poor, with a 1-year survival rate of less than 55% and a 3-year survival rate of less than 25%. The average survival time of patients with metastasis is less than 3 months. The postoperative survival time of patients with this disease is closely related to the T stage at the time of diagnosis. The median survival times of patients with T1-2 and T3-4 tumors are 23.9 months and 16.0 months, respectively [2]. Early diagnosis is therefore particularly important for survival.

CONCLUSION

Primary LCNEC of the bladder is an extremely rare malignant bladder tumor. Immunohistochemical examination is indispensable for diagnosing LCNEC of the bladder. For patients whose tissue biopsy has been obtained before surgery, we recommend immunohistochemical examination to determine the source of the tumor. Early diagnosis and multimodal treatment are highly important for prolonging patients' survival.

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Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Figure Legends

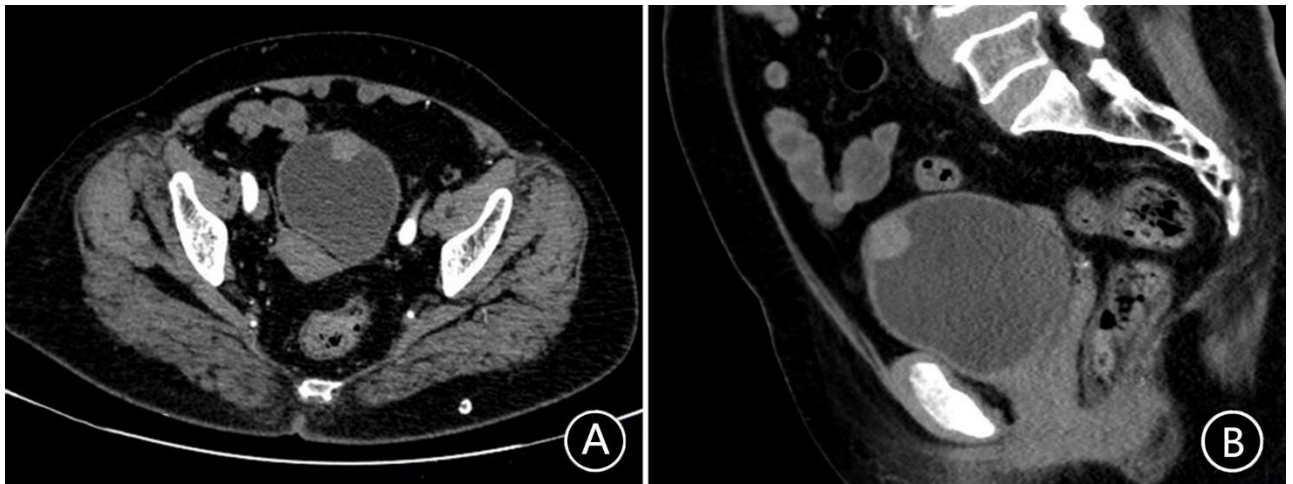


Figure 1 Preoperative pelvic contrast-enhanced CT. Pelvic contrast-enhanced CT showing a localized tumor on the anterior wall of the bladder measuring approximately 1.68 cm×1.72 cm×1.74 cm, which was slightly enhanced. A: transverse plane. B: Sagittal plane.

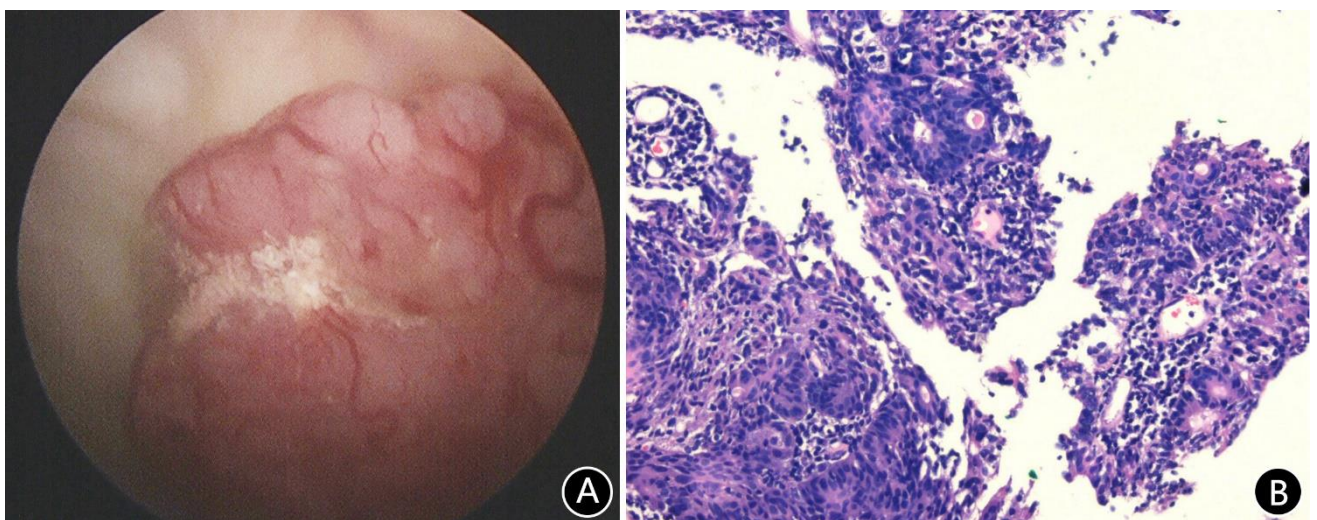


Figure 2 Cystoscopy and pathological examination of tissue samples obtained after biopsy. A: Cystoscopy showed that there was a 2 cm × 2 cm-sized spherical

pink mass on the anterior wall of the bladder with tortuous blood vessels on the surface. B: Pathological examination after biopsy with hematoxylin and eosin staining showed cells with pronounced atypia, large and differently shaped cells and nuclei, and deeply stained nuclei. Bladder malignant tumor was considered after pathological examination ($\times 200$).

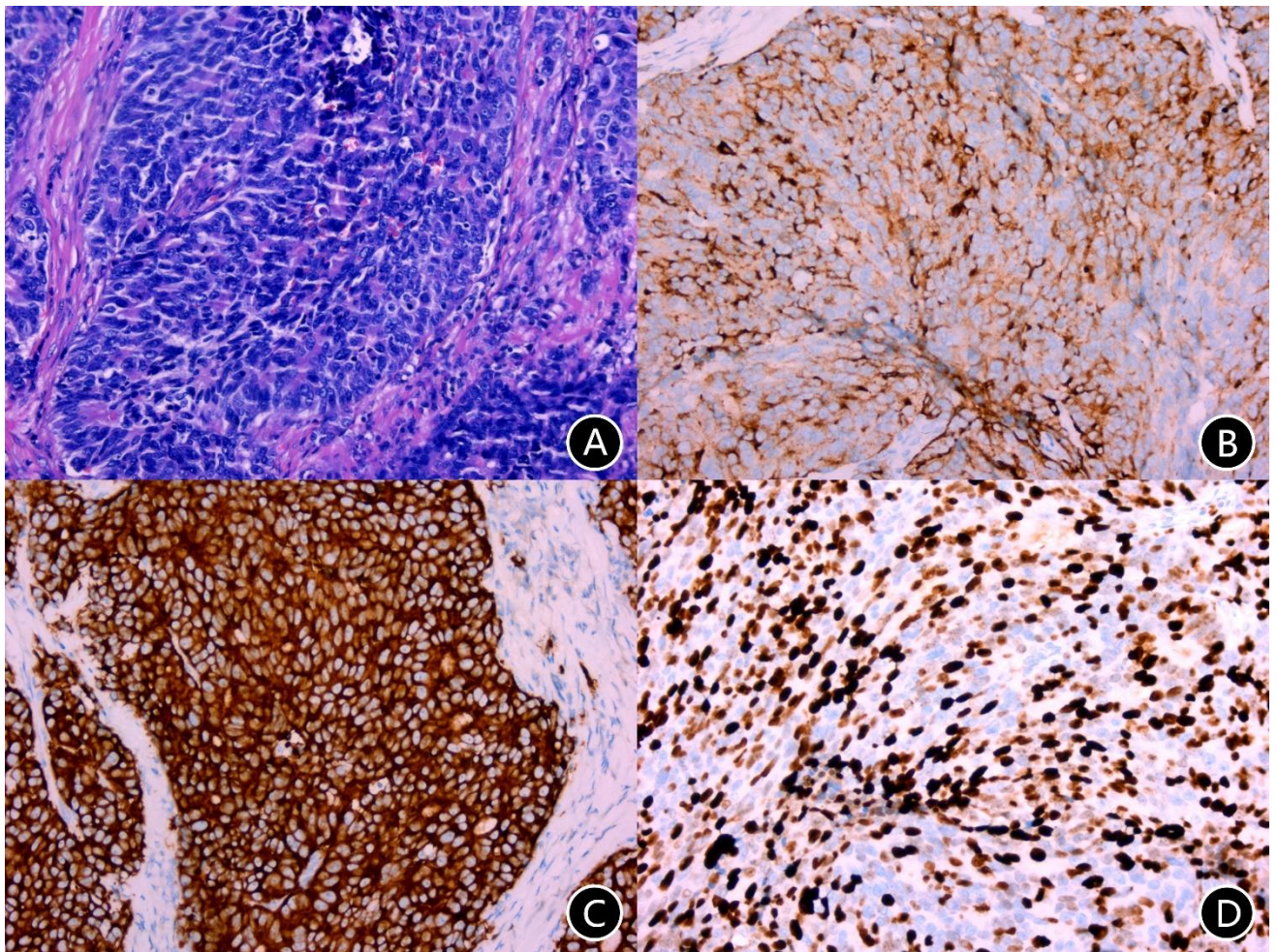


Figure 3 Pathological examination and immunohistochemical staining after surgery. A: Hematoxylin and eosin staining found tumor cells had large nuclei, coarse granular chromatin, visible nucleoli, mitotic figures, and palisade-like structures ($\times 200$). B: Chromogranin A was positive ($\times 200$). C: Synaptophysin was positive ($\times 200$). D: The proliferative index Ki67 was 70% ($\times 200$).