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Research Article

Neuroendocrine carcinoma of the cervix: A comprehensive clinicopathologic study and literature review

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ABSTRACT

Aims: Neuroendocrine carcinoma of the cervix (NECC) is a rare variant of cervical cancer. This study aims to investigate the clinicopathological features and prognosis of NECC.
Methods: A retrospective analysis was conducted on twenty-one patients diagnosed with NECC between May 2008 and September 2021 at Peking University People's Hospital. The study involved histopathological examination, immunohistochemistry, ThinPrep cytology test (TCT), and high-risk HPV hybrid capture 2 (HC2) assay. Follow-up was conducted through telephone interviews and medical records for a range of 3–160 months, with an average follow-up period of 49.8 months.
Results: The average age of the patients was 48.6 years (range: 33–69 years). Seventeen patients were diagnosed with neuroendocrine carcinoma through biopsy. Nine cases underwent TCT and HC2 tests before biopsy, and TCT results of four cases showed high-grade squamous intraepithelial lesion (HSIL). High-risk HPV(HR-HPV) positive was detected in seven cases. The cancer cells exhibited consistent morphological features, including sparse cytoplasm, intensely stained nuclei, and extensive neoplastic necrosis. Thirteen cases were classified as pure NECC (61.9%), while eight cases were mixed types (38.1%). Three cases were associated with squamous cell carcinoma (SCC), and five cases were associated with adenocarcinoma. Prognosis varied significantly among these subtypes ($p < 0.05$). The overall survival rate in the follow-up period was 66.7% (12/18).
Conclusions: NECC is an extremely rare and highly aggressive tumor with a poor prognosis, particularly in cases of mixed histology. It is strongly associated with HPV infection. TCT and HPV testing significantly enhance the detection rate before the biopsy. The diagnosis of NECC relies on histological and immunohistochemical examinations. This study provides valuable clinical observations on NECC and emphasizes the importance of early detection and accurate diagnosis for improved patient outcomes.

1. Introduction

Cervical cancer is a prevalent malignant tumor affecting the female reproductive system. Squamous cell carcinoma (SCC) is the most common type, accounting for over 90% of cases.[1] However, primary neuroendocrine carcinoma of the cervix (NECC) is rare, as neuroendocrine cancer is typically found in the lungs and digestive tracts. NECC represents only approximately 1%~1.5% of all primary cervical cancers.[2,3] Despite its rarity, NECC is an aggressive histological variant, sharing clinical manifestations similar to SCC and adenocarcinoma of the cervix. Detecting early-stage cervical neuroendocrine cancer poses challenges.[4] Nevertheless, NECC exhibits distinct biological characteristics compared to SCC or adenocarcinoma, demonstrating malignancy, aggressiveness, and poor outcomes. Immunohistochemical markers such as Syn, CgA, and CD56 are frequently employed in diagnosing NECC.[5] Additionally, p16, which is often positive in NECC, may be associated with oncogenic HR-HPV.[6] NECC is more likely to involve lymphovascular space invasion (LVSI) and spread to regional lymph nodes during the initial diagnosis.[7]

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This study retrospectively analyzes the clinical manifestations, histopathological features, immunohistochemistry, and clinicopathological characteristics of NECC, while also reviewing relevant literature to emphasize the tumor's pathological characteristics, diagnostic criteria, and outcomes. The aim is to enhance understanding of this tumor and prevent missed or delayed diagnoses.

2. Materials and methods

2.1. Clinical data

Twenty-one cases of NECC were retrospectively analyzed at Peking University People's Hospital, covering the period from May 2008 to September 2021. The study involved two experienced gynecological pathologists who reviewed all cases. After conducting general check-ups, laboratory examinations, and follow-ups, the cases were confirmed to be primary NECCs.

The pathological diagnosis of NECC was based on the 2020 World Health Organization (WHO) classification.[8] The inclusion criteria for SCNEC small-cell neuroendocrine carcinoma (SCNEC) were as follows: The tumor exhibited sheets of highly atypical cells with scant cytoplasm. The nuclei were ovoid to slightly spindled, showing hyperchromatic and dispersed chromatin with inconspicuous nucleoli. The cells were arranged in solid, nested, trabecular, pseudo-glandular, and rosette-like patterns. The criteria of Large cell neuroendocrine carcinoma (LCNEC) is a high-grade cacinoma composed of large cells with neuroendocrine differentiation. If a single neuroendocrine tumor is detected, it can be diagnosed as pure NECC. However, if two tumor components are simultaneously detected, with each component accounting for at least 10% of the tumor, the diagnosis would be classified as mixed-type NECC.

2.2. Cytology examination

Before the biopsy, cytologic slides were prepared using the TCT (Hologic, Bedford, MA) method. All cytologic findings were reported in accordance with The Bethesda System terminology.

2.3. HPV test

Nine patients underwent an HR-HPV test using HC2 (Digene, Gaithersburg, MD, USA). A positive result was defined as HPV DNA equal to or greater than 1 pg/ml.

2.4. Immunohistochemistry (IHC)

All pathological specimens were fixed with 4% neutral formaldehyde and underwent dehydration, soaking, embedding in paraffin, and staining with hematoxylin-eosin. Immunohistochemical tests were conducted following the manufacturer's protocols. The primary antibodies used were as follows: p63 (UMAB4, ZSGB, 1:100); p16 (E6H4, Roche, Roche Benchmark Ultra; 1:100); Estrogen receptor (ER) (6F11, Leica, RTU; 1:100); Progesterone receptor: PR (16, Leica, RTU; 1:100); CK7 (EP16, ZSGB,1:100); CK20 (EP23, ZSGB, 1:100); CDX-2 (EP25, ZSGB,1:200); Ki-67 (EP5, ZSGB,1:200); Synaptophysin (Syn) (UMAB112, ZSGB,1:100); Chromogranin ：CgA (EP38, ZSGB,1:100), CD56 (UMAB83, ZSGB, 1:100), CKpan (AE1/AE3, ZSGB, 1:200). Epithelial membrane antigen: EMA (UMAB57, ZSGB,1:100),CEA (12-40-10, ZSGB, 1:100), Thyroid Transcription Factor-1:TTF1(SPT24, ZSGB, 1:100), Retinoblastoma protein: RB (13A10, ZSGB, 1:100),p53 (DO-7, ZSGB,1:100). Positive staining was observed as brown-yellow color. Ki-67, TTF1, CDX2, Rb, and p53 positive sites were located in the nucleus, while CKpan, CK7, CK20, Syn, CD56, and CgA positive cells were observed in the cytoplasm.

2.5. Statistical analysis

The data analysis was performed using IBM Corp.'s SPSS statistical v25.0 software. Fisher's exact tests were utilized to analyze the clinicopathological features between the pure and mixed-type groups. A significance level of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Clinicopathological features

The study cohort consisted of twenty-one patients who were diagnosed with primary NECC. The average age of the patients was 48.6 years, ranging from 33 to 69 years. The presenting symptoms varied among the patients; eleven had vaginal bleeding, two had vaginal discharge, five presented with a cervical mass, and the remaining patients were asymptomatic. Nine patients underwent TCT before the biopsy. Among them, five had negative results, and four had positive results, indicating high-grade squamous intraepithelial lesions (HSILs) (Table 1). Cytology examination revealed scattered or clumpy distribution of small cells with high cytoplasm, hyperchromatic nuclei, inconspicuous nucleoli, typical mitotic images, and apoptotic bodies (Fig. 1). Among the nine patients who underwent HPV testing, seven tested positive for HR-HPV, while two tested negative (Table 1). Seventeen patients received a diagnosis of neuroendocrine carcinoma through biopsy, while four were diagnosed with other tumors upon radical resection. None of the patients received chemotherapy before the diagnosis. Surgical interventions included extensive hysterectomy with bilateral adnexa and either pelvic lymph node or para-aortic lymph node resection for sixteen patients, hysterectomy with bilateral adnexa resection for four patients, and cervical conical resection for one patient. Five patients underwent neoadjuvant chemotherapy before surgery, while twelve received it after surgery.

3.2. Histopathological features

On gross examination, most cases of NECC exhibited exophytic polyps or cauliflower-like patterns, resembling other types of cervical carcinomas. The maximum tumor diameter ranged from 2.5 to 7.0 cm. The cut surfaces often appeared white or gray-red, with a solid consistency accompanied by areas of hemorrhage and necrosis (Fig. 2). In twenty cases, tumor cells were microscopically observed in adenoid, band-like, clustered, or diffuse patterns, while the surface epithelium was not invaded. The morphology of the cancer cells exhibited consistency, with sparse cytoplasm and blue-stained nuclei, along with extensive neoplastic necrosis. These findings were consistent with SCNEC (Fig. 3A–D). In one patient, the tumor cells appeared relatively larger, and organ-like structures were observed, leading to a diagnosis of large cell neuroendocrine carcinoma (LCNEC) (Fig. 4A–D). ER, and PR were negative in all 21 cases. Syn, CgA, and CD56 showed varying degrees of positivity, with rates of 85.7% (18/21), 42.9% (9/21), and 85.7% (18/21), respectively. Epithelial markers, CKpan, EMA, and CEA, showed positivity rates of 90.5% (19/21), 61.9% (13/21), and 38.1% (8/21), respectively. The positivity rate of p16 was 81% (17/21), Rb loss was observed in 95.2% (20/21) of cases, and p63 showed positivity in 42.9% (9/21) of cases. Mutated p53 expression was present in 38.1% (8/21) of cases, while wild-type p53 expression was observed in 61.9% (13/21) of cases. The positive rates of TTF1 and CDX2 were 52.4% (11/21) and 38.1% (8/21), respectively. CK7 showed a positive expression in all 21 cases (100%), while CK20 was negative in all 21 cases (Table 2).

Clinicopathological characteristics of pure and mixed cancers were compared. Thirteen cases were classified as pure NECC (61.9%), with two cases accompanied by HSIL and one case associated with adenocarcinoma in situ (AIS). Eight cases were classified as mixed types (38.1%), with three cases accompanied by SCC and five cases accompanied by adenocarcinoma. There were no significant differences between the two groups regarding age, clinical 2018 FIGO stage, tumor size, LVSI, or lymph node metastasis, as determined by Fisher's test ($p > 0.05$). However, there was a statistically significant difference in

Table 1
Clinicopathological features with 21 cases of NECC.

Patient ID	Clinical feature	FIGO Stage (2018)	TCT	HPV	Biopsy diagnosis	Tumor size (cm)	Pathologic diagnosis	Accompanying diagnosis	Operation	Prognosis
1	vaginal bleeding	Ib1	N	N	SCNEC	3.0 × 2.5	SCNEC	No	EHBAPLN	alive
2	vaginal bleeding	Ib1	/	/	SCNEC	1.5 × 1.8	SCNEC	ACC	EHBAPLN	death
3	vaginal discharge	Ib1	/	/	SCNEC	2.5 × 0.9	SCNEC	SCC	EHBAPLN	death
4	vaginal bleeding	Ib1	/	/	SCNEC	1.5 × 1.3	SCNEC	ACC	HBA	Lost
5	cervical mass	Ib1	N	P	SCNEC	2.5 × 1.8	SCNEC	No	EHBAPLN	alive
6	vaginal bleeding	Ib1	N	N	SCNEC	1.5 × 0.8	SCNEC	No	EHBAPLN	alive
7	vaginal bleeding	Ib1	HSIL	P	HSIL	0.8 × 0.5	SCNEC	SCC	EHBAPLN	alive
8	vaginal bleeding	Ib1	/	/	HSIL	2.2 × 0.9	SCNEC	No	HBA	alive
9	vaginal discharge	Ib1	HSIL	P	SCNEC	2.6 × 2.1	SCNEC	SCC	EHBAPLN	alive
10	vaginal bleeding	Ib1	/	/	SCNEC	1.7 × 0.6	SCNEC	AIS	EHBAPLN	alive
11	vaginal bleeding	Ib2	/	/	SCNEC	2.3 × 1.8	SCNEC	ACC	EHBAPLN	death
12	cervical mass	Ib1	N	N	SCNEC	2.0 × 0.8	SCNEC	No	CCR	Lost
13	cervical mass	Ib1	N	P	SCNEC	2.0 × 1.0	SCNEC	No	EHBAPLN	alive
14	asymptomatic	Ib1	/	/	HSIL	0.5 × 0.3	SCNEC	No	EHBAPLN	alive
15	vaginal bleeding	Ib1	/	/	SCNEC	1.8 × 1.6	SCNEC	ACC	EHBAPLN	death
16	vaginal bleeding	Ib1	/	/	SCNEC	0.9 × 0.5	SCNEC	No	HBA	Lost
17	vaginal bleeding	Ib1	/	/	SCNEC	2.0 × 0.7	SCNEC	ACC	EHBAPLN	death
18	asymptomatic	Ib1	/	/	HSIL	0.7 × 0.5	LCNEC	No	EHBAPLN	alive
19	cervical mass	Ib1	HSIL	P	SCNEC	1.8 × 0.6	SCNEC	HSIL	EHBAPLN	alive
20	vaginal bleeding	Ib1	/	/	SCNEC	2.0 × 0.7	SCNEC	No	HBA	alive
21	cervical mass	Ib2	HSIL	P	SCNEC	2.5 × 2.0	SCNEC	HSIL	EHBAPLN	death

Note: SCNEC: small cell neuroendocrine carcinoma; LCNEC:large cell neuroendocrine carcinoma N: negative; P: positive; EHBAPLN: extensive hysterectomy-bilateral adnexa pelvic lymph node; HBA: hysterectomy bilateral adnexa resection; CCR: cervical conical resection; ACC: adenocarcinoma of cervix; SCC: squamous cell carcinoma; HSIL: high-grade squamous intraepithelial lesion; AIS: adenocarcinoma in situ.

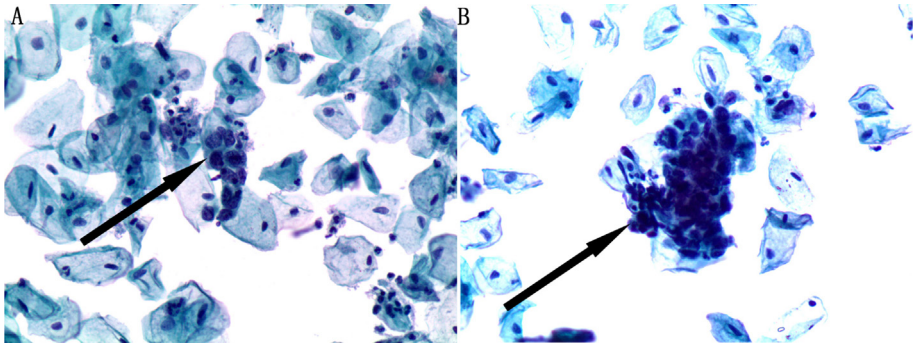

Fig. 1. Cytological features of SCNEC accompanied by HSIL. (A) The image shows a single detached cell or cluster of several cells. The cells are small in size with a high nuclear-to-cytoplasmic ratio. The nuclei appear hyperchromatic and coarse-grained (black arrow). (B) The image displays a larger cluster of several cells (black arrow).

prognosis ($p < 0.05$) (Table 3).

3.3. Follow-up

Telephone and medical records were followed up for a period ranging from 3 to 160 months, with an average follow-up duration of 49.8 months. Out of the twenty-one patients included in the study, twenty underwent surgery. Among them, six patients passed away, and three were lost to follow-up. The remaining twelve patients were alive and showed no signs of recurrence or metastasis. Therefore, the overall survival rate was calculated as 66.7% (12 out of 18 patients for whom the survival status was available).

4. Discussion

Cervical neuroendocrine tumors originate from neuroendocrine cells. The first report of this disease dates back to 1957 by Reagan et al.[9] Primary neuroendocrine cervical tumors are exceptionally rare, accounting for only 1%~1.5% of all primary cervical tumors.[2] In 1997, the American Society for Cancer Research categorized NECC into typical carcinoid, atypical carcinoid, LCNEC, and SCNEC.[10] The 2014 WHO classification of female reproductive system tumors[1] recommended adopting the classification system used for the gastrointestinal tract and pancreatic neuroendocrine tumors. Cervical neuroendocrine tumors were classified as low-grade or high-grade neuroendocrine carcinoma. Low-grade tumors encompassed low-grade neuroendocrine tumors grades 1 and 2, which were equivalent to typical carcinoid and atypical carcinoid tumors, respectively.

High-grade neuroendocrine carcinomas were classified as SCNEC and LCNEC, corresponding to small-cell and large-cell carcinomas, respectively. However, in the 2020 WHO classification of female reproductive system tumors, all neuroendocrine neoplasms were consolidated into one chapter, and the distinction between low-grade neuroendocrine tumors and high-grade neuroendocrine carcinomas, except for ovarian cancer, was not recommended.[8] Typical carcinoid and atypical carcinoid tumors are extremely rare among these four tumor types, with SCNEC being the

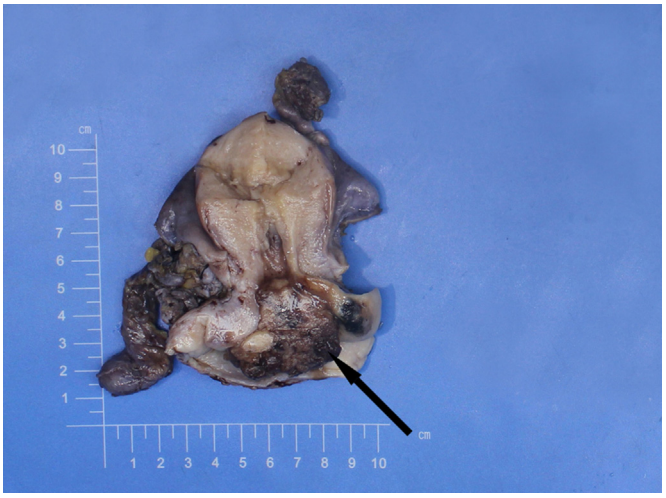

Fig. 2. Representative image of SCNEC, demonstrating exophytic polyps.

most common variant. To date, only approximately seventeen cases of LCNEC have been reported.[11] In the present study, SCNEC was the predominant type, with twenty cases identified, while only one case of LCNEC was observed. No cases of typical carcinoid or atypical carcinoid tumors were found.

According to the WHO classification criteria for neuroendocrine lung tumors, if two tumor components are simultaneously detected, with each component accounting for at least 10% of the tumor, it can be diagnosed as mixed neuroendocrine carcinoma. If the neuroendocrine component is less than 10%, the diagnosis is considered SCC or adenocarcinoma with focal neuroendocrine differentiation. The mixed components may include SCC and adenocarcinoma.[12] In some cases, SCC and adenocarcinomas of the cervix, along with their precursor lesions, may coexist with neuroendocrine carcinomas. However, glandular lesions appear to be more common.

In the present review, thirteen cases of pure NECC and eight cases of mixed types of neuroendocrine cancers were examined. Among the mixed types, three cases were accompanied by SCC, and five cases were associated with adenocarcinoma. Among the pure NECCs, two were associated with HSIL, and one was associated with AIS. The clinicopathological characteristics of pure and mixed types were compared. No significant differences were observed between the two groups in terms of age, clinical FIGO stage, tumor size, LVSI, or lymph node metastasis ($p > 0.05$). However, the mixed group had a significantly worse prognosis ($p < 0.05$). Thus, there were no notable differences in clinicopathological features between the two groups.

The findings of this study suggest that attention should be given to the mixed type of neuroendocrine carcinoma. However, it is important to note that the number of cases in this study was limited, and larger sample sizes are required to draw more definitive conclusions.

The clinical manifestations of NECC vary and are similar to those of other types of cervical carcinomas. These manifestations include vaginal bleeding, discharge, or the detection of distinct cervical masses.[1] In our cohort of patients, the common symptoms observed were irregular vaginal bleeding and discharge, which lack specificity. Neuroendocrine-related symptoms were not observed in most cases, although they occasionally accompanied symptoms of carcinoid syndrome, which was rare. Only a small number of low-grade neuroendocrine tumors produce peptide hormones such as gastrin-releasing peptides and serotonin. In rare cases, ectopic hormone production can cause symptoms. However, most neuroendocrine carcinomas do not secrete peptide hormones, and even if they do, the activity is either negligible or insufficient to produce symptoms.[13] In our cohort, no neuroendocrine symptoms were found.

The pathogenesis of cervical neuroendocrine neoplasms is not fully understood. Some low-grade cervical neuroendocrine neoplasms appear to originate from neuroendocrine cells located in the cervical glandular and squamous epithelium.[14] NECC is associated with HPV infection. Similar to SCC, HR-HPV can be detected in the majority of NECC.[15,16] HPV infection plays a crucial role in the development of NECC.[17] In a recent meta-analysis by Castle et al. HPV infection data from 403 cases of SCNEC and 45 cases of LCNEC were analyzed.[16] They found that more

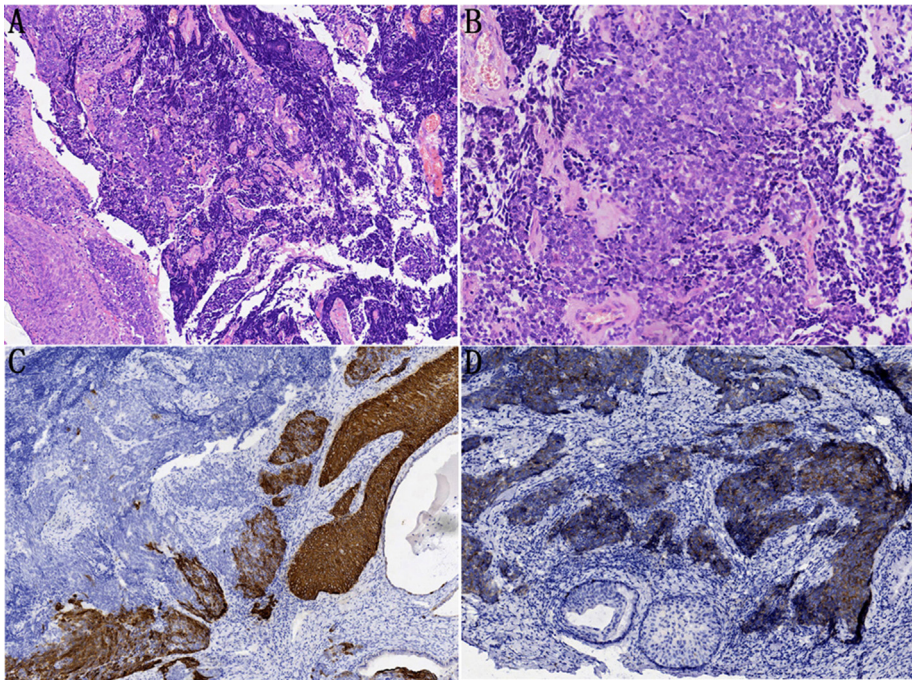

Fig. 3. (A) HE staining of SCNEC, illustrating carcinoma cells arranged in diffuse patches (100×). (B) At higher magnification, the cancer cells exhibit hyperchromatic nuclei, scant cytoplasm, abundant mitotic activity, and partial extrusion. The nuclear-to-cytoplasmic ratio is high (200). (C) Immunohistochemical staining of CK5/6. SCNEC cells show negative staining, while HSIL cells show positive staining (200×). (D) Immunohistochemical staining of Syn. SCNEC cells exhibit positive staining, while HSIL cells show negative staining (200×).

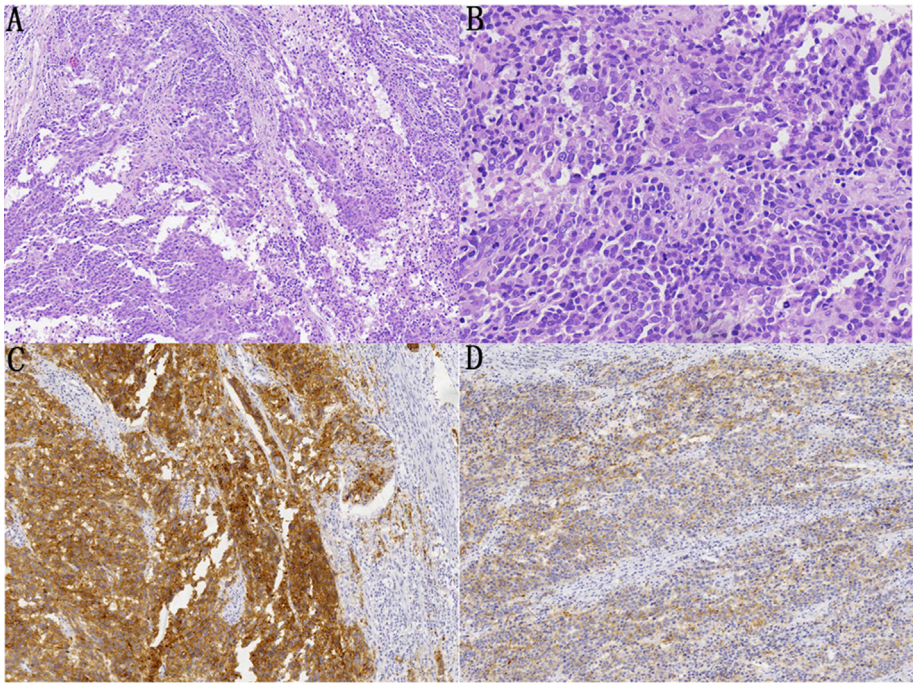

Fig. 4. (A) HE staining of LCNEC showing carcinoma cells arranged in organ-like structures (HE 100×). (B) At high magnification, cancer cells had prominent nucleoli and abundant cytoplasm(HE 200×). (C) Immunohistochemical staining of Syn-positive LCNEC specimens (HE 200×). (D) Immunohistochemical staining of CgA-positive LCNEC specimens (HE 100×).

Table 2
Immunohistochemical expression of all cases.

Patient ID	CKpan	EMA	CEA	CK7	CK20	ER	PR	P16	Syn	CgA	CD56	CDX2	TTF1	P53	Rb	Ki67(%)
1	+	+	–	+	–	–	–	+	+	+	+	+	+	W	–	30
2	+	+	+	+	–	–	–	+	+	+	+	–	+	M	–	50
3	+	+	–	+	–	–	–	+	+	–	+	–	–	W	–	20
4	+	–	–	+	–	–	–	+	+	–	+	+	–	W	–	60
5	+	+	+	+	–	–	–	–	+	+	+	+	+	W	–	50
6	+	+	–	+	–	–	–	+	+	+	–	+	–	M	–	50
7	+	–	+	+	–	–	–	+	–	+	+	–	+	W	–	40
8	+	–	–	+	–	–	–	+	+	–	+	+	+	M	–	60
9	+	+	+	+	–	–	–	+	+	–	+	–	–	W	–	60
10	+	–	+	+	–	–	–	–	+	+	+	–	+	W	–	30
11	+	+	–	+	–	–	–	+	+	+	+	+	–	W	–	70
12	+	–	–	+	–	–	–	+	+	–	+	–	–	W	–	40
13	+	+	+	+	–	–	–	+	+	+	+	–	+	M	+	50
14	+	+	–	+	–	–	–	+	+	–	–	–	–	W	–	30
15	+	+	–	+	–	–	–	–	+	+	–	–	+	W	–	50
16	+	+	+	+	–	–	–	+	–	+	+	+	–	M	–	70
17	+	–	–	+	–	–	–	+	–	+	+	–	+	M	–	60
18	+	+	–	+	–	–	–	+	+	–	+	–	–	M	–	60
19	–	+	+	+	–	–	–	–	+	–	+	–	–	M	–	40
20	+	–	–	+	–	–	–	+	+	+	+	+	+	W	–	40
21	–	–	–	+	–	–	–	+	+	–	+	–	+	W	–	80

Note: W:wild type; M:mutated; Ckpan: cytokeratin; EMA: epithelial membrane antigen; CgA: chromogranin; Syn: synaptophysin; ER: Estrogen receptor; PR: Progesterone receptor; TTF1: Thyroid Transcription Factor-1; RB: retinoblastoma protein.

than 80% of the cases were positive for HR-HPV. The most prevalent HPV subtypes were HPV 18 and 16. The authors concluded that the E6 protein of HPV binds to the wild-type p53 protein, causing its functional loss, which may be the underlying cause of NECC. Some cervical SCNECs have recurrent genetic alterations involving the MAPK, PI3K/AKT/mTOR, and p53/BRCA pathways.[18] Genetic studies have shown that SCNECs, similar to small-cell carcinoma of the lung, frequently exhibit abnormalities in the 3p alleles and occasional deletions in 9p21,[1] suggesting shared etiological factors for these tumors. In the present study, HR-HPV tests were performed on nine patients, with seven testing positive (7/9, 77.8%).

TCT for NECC is not sufficiently sensitive or specific. In the present study, the detection rate of NECC using TCT was significantly lower than that of SCC. Among the nine patients who underwent TCT before the biopsy, five were negative, and four cases were positive and diagnosed with HSIL. NECC cells were not detected by TCT. Cytologically, the findings typically included single detached cells or clusters of several cells. The cells exhibited size variation, but they tended to be small, with

Table 3
Comparison of clinicopathological features with NECC of pure and mixed type.

	Pure NECC	Mix type NECC	*P value*
Cases	13	8	
Ages			0.673
≥50	5	4	
< 50	8	4	
Tumor size (cm)			
≤4	12	7	1.000
> 4	1	1	
FIGO Stage (2018)			1.000
≤ Ib1	12	7	
> Ib2	1	1	
Pathologic diagnosis			/
Accompanying diagnosis	AIS(1 case), HSIL (2 cases)	SCC(3 cases), ACC(5 cases)	
LVSI			0.531
Negative	12	6	
Positive	1	2	
Lymph node metastasis			0.381
Yes	0	1	
No	13	7	
Prognosis			0.043
alive	9	3	
Die	1	5	

Note: NECC: Neuroendocrine carcinoma of the cervix; ACC: Adenocarcinoma of the cervix; SCC: squamous cell carcinoma; HSIL: high-grade squamous intraepithelial lesion; AIS: adenocarcinoma in situ; LVSI: lymphovascular space invasion; FIGO: the International Federation of Gynecology and Obstetrics.

a high nuclear-to-cytoplasmic ratio. The nuclei appeared hyperchromatic and coarse-grained, and sometimes a "squeezed" phenomenon was observed. These findings suggest that in some cases, HSIL is diagnosed. Due to the small size of the HSIL cells and the high nuclear-to-cytoplasmic ratio, the chromatin of the nucleus appears coarse. Another possible explanation is that TCT only sampled the superficial part of the lesion, while neuroendocrine carcinoma can coexist with SCC or HSIL lesions, which can only be detected using microscopy.

Among the four positive cases, the final pathological diagnosis revealed that two cases were associated with SCC, and two cases were associated with HSILs. The five negative cases were pure NECC, which were not detected by TCT. Since our focus was primarily on identifying infiltrating stromal elements of pure neuroendocrine cancer cells, only cases that presented with simultaneous pathological changes could potentially improve the cytological detection rate. The cases in this particular group fell into the latter category. These findings suggest that regardless of TCT results, increasing the detection of HR-HPV (especially in symptomatic patients) will significantly improve the diagnostic rate of early NECC. Combining TCT with HR-HPV testing could significantly enhance the detection rate of NECC before resorting to a biopsy. In this study, 17 patients were ultimately diagnosed with NECC through cervical biopsy. Four cases were initially missed by the biopsy but were later diagnosed based on subsequent radical resection specimens. This discrepancy could be attributed to the superficial sampling site during colposcopy, insufficient sampling specimens, or limited experience of the pathologists involved.

Immunohistochemical staining is a valuable supportive diagnostic method for NECC. It involves the use of epithelial tissue markers such as Ckpan and EMA, as well as neuroendocrine markers including CgA, Syn, CD56, and NSE. Among these markers, CD56 has the highest sensitivity, with positivity rates reaching up to 80%.[19] However, its specificity is relatively low, as CD56 staining can also be observed in non-neuroendocrine carcinomas, including lymphoma.[20] Syn is generally more sensitive than CgA, while CgA and NSE exhibit lower sensitivities. In the present study, Syn and CD56 were positive in 18 cases (85.7%, 18/21), while CgA was positive in nine cases (42.9%, 9/21), with more diffuse positive staining observed than focal positive staining. These findings align with previously reported positivity rates.[5] CgA showed strong specificity but limited sensitivity, resulting in a relatively low positivity rate in this study.

$p16^{INK4a}$ staining was diffusely or focally positive in 17 cases (81%, 17/21), consistent with the findings of some literature.[21] Their study indicated that $p16^{INK4a}$ overexpression was significantly associated with the presence of HR- HPV strains in NECC and was not correlated with p53 or NRAS mutations. TTF-1 was frequently positive in 11 cases (52.4%, 11/21). The positive rate of TTF-1 is 85% in small-cell lung cancer and ranges from 40% to 50% in large-cell lung cancer and extrapulmonary NECC.[22] However, the TTF-1 positivity rate in NECC is typically lower than that in pulmonary NECC. The differential immunohistochemical expression of TTF-1 in cervical and pulmonary NECC may be attributed to the distinct pathogenesis of these diseases.[23] Nonetheless, TTF-1 staining does not hold diagnostic value in differentiating between cervical and pulmonary primary tumors.[6,24]

Loss of Rb expression was observed in 95.2% (20/21) of cases, while p53 mutated expression was found in 38.1% (8/21) of cases. The loss of Rb expression and mutated p53 expression at this early stage suggest genetic and epigenetic damage contributing to the development of NECC.[25] Loss of Rb expression by IHC may serve as an ancillary marker supporting the diagnosis of NECC in routine practice, as it is a molecular genetic hallmark of small cell lung cancer and appears to hold true for NECC as well.[26]

The main differential diagnoses for NECC include poorly differentiated adenocarcinoma, SCC of the cervix, and metastatic neuroendocrine carcinoma. Occasionally, poorly differentiated carcinomas may exhibit focal regional neuroendocrine differentiation, but this should not exceed 10% of the tumor. Otherwise, mixed NECC should be considered. Neuroendocrine markers (such as Syn, CgA, and CD56) in poorly differentiated carcinomas often show variable local positivity or negativity.[24] Since NECC is histologically indistinguishable from other neuroendocrine tumors, it is crucial to rule out the possibility of metastasis from another site. Similar to pulmonary neuroendocrine tumors, NECCs are aggressive tumors with a high propensity for metastasis.[7] Due to their rarity, they are often misdiagnosed, and standardized treatment regimens are lacking. Therefore, treatment options have been adapted from lung neuroendocrine tumors. Early-stage patients (Stage I-IIA) are typically treated with radical surgery and chemotherapy, while intermediate-to-advanced stage patients (Stage IIB-IV) receive radiation and chemotherapy.[27] Factors such as advanced clinical stage, lymph node metastasis, and depth of tumor infiltration have been identified as independent prognostic factors.[28] NECC is associated with poor prognosis, with six patients in this study succumbing to the disease. The literature showed that the 5-year survival rate is extremely low, much lower than that of SCC.

In conclusion, NECC is an extremely rare primary tumor characterized by aggressive behavior and poor prognosis, particularly in the case of mixed types. The tumor is associated with HPV infection. The combined use of TCT and HPV testing can significantly improve the detection rate before resorting to a biopsy. The pathological diagnosis relies on histological examination and immunohistochemistry.

Contributions

ZX, LM, ZG, and SD were involved in performing experiments. ZX contributed to data acquisition. ZX, SD, ZG, and LM were involved in conducting the experiments. ZX contributed to data acquisition and drafted and wrote the manuscript. DS designed and supervised the study. ZG was responsible for the follow-up of cases. All authors have read and approved the final manuscript.

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Ethics approval and consent to participate

This retrospective study received approval from the Ethics Committee of Peking University People's Hospital (Beijing, China). Given the retrospective nature of the study and the anonymous analysis of preexisting data, the need to obtain further written informed consent from individual patients was waived by the Ethics Committee.

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Patient consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no competing interests.

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