

Cytological features of small cell neuroendocrine carcinoma of the uterine cervix

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Abstract. Small cell neuroendocrine carcinoma (SCNEC) of the cervix is rare. Cytological examination is a valuable tool for detecting cervical lesions; however, due to the rarity of cervical SCNEC, its accurate diagnosis remains challenging. In some cases, cervical SCNEC coexists with other non-neuroendocrine components, forming what is known as combined SCNEC. The cytological features of combined SCNEC have not been well-documented. The present study retrospectively analysed the clinicocytological features of cervical SCNEC, including combined SCNEC. A total of six patients diagnosed with cervical SCNEC (three with pure SCNEC, two with combined SCNEC and one with carcinosarcoma) who underwent preoperative cervical cytological examination and were diagnosed with SCNEC through biopsy and/or surgical resection were included in the current study. The median patient age was 55 years, and the combined SCNEC cases included both SCNEC and adenocarcinoma components. Retrospective cytological examination revealed that cervical specimens from all six patients contained SCNEC components, characterised by clusters of small round neoplastic cells with round to oval nuclei, fine chromatin, and inconspicuous nucleoli in a necrotic or inflammatory background. Among the two patients with combined SCNEC, one exhibited an adenocarcinoma component, whereas the patient with carcinosarcoma had a sarcomatous component. These findings suggested that most

cases of SCNEC can be detected in cervical cytological specimens. Additionally, other combined carcinoma components may be identified. Notably, careful cytological examination may facilitate the accurate diagnosis of cervical SCNEC, enabling timely treatment for this aggressive malignancy.

Introduction

Small cell neuroendocrine carcinoma (SCNEC) is a highly aggressive malignancy. Although it most commonly arises in the lungs, its occurrence in the gynaecological tract is rare (1). When SCNEC does affect gynaecological organs, the uterine cervix is the most frequently involved site (1). Cervical SCNEC accounts for <1% of all gynaecological malignancies and <5% of all cervical cancers (2,3). Compared to cervical squamous cell carcinoma (SCC) and adenocarcinoma, cervical SCNEC has a significantly worse prognosis (1-4). Therefore, early detection and multimodal therapy are crucial for improving patient outcomes.

Cytological examination is one of the gold standards for detecting cervical lesions because it is convenient, minimally invasive, and cost-effective (5). Thus, cytological diagnosis plays a crucial role in the early detection of cervical SCNEC. However, due to its rarity, diagnosing SCNEC cytologically can be challenging. In one study, only one out of 13 patients was suspected of having cervical SCNEC based on cytological findings (6). In another cytological series, none of the patients were accurately diagnosed with cervical SCNEC (7). Instead, patients were often misdiagnosed with squamous lesions, which are far more common (6,7). The cytological features of cervical SCNEC are similar to those of other organs, such as the lungs. These features include loose cohesive clusters and/or single small, round neoplastic cells with a high nuclear-to-cytoplasmic ratio, oval-to-round nuclei with finely granular chromatin and inconspicuous nucleoli, nuclear moulding in a necrotic background (6-10). Moreover, cervical SCNEC may coexist with other histological types of non-neuroendocrine neoplasms, including squamous intraepithelial lesions (SIL), SCC, and adenocarcinoma (11-15). The presence of these additional components may lead to a misdiagnosis of SCNEC components in cytological specimens, as

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Abbreviations: HPV, human papillomavirus; SCC, squamous cell carcinoma; SCNEC, small cell neuroendocrine carcinoma; SIL, squamous intraepithelial lesion

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SIL, SCC, or adenocarcinoma components can overshadow the SCNEC features (16-19).

This study retrospectively analysed the cytological features of cervical SCNEC, including combined SCNEC, and discussed the clinicocytological features of this rare tumour, with a focus on identifying coexisting carcinoma components.

Materials and methods

Patient selection. Patients diagnosed with cervical SCNEC based on pathological examination of biopsy and/or surgically resected specimens at Osaka Medical and Pharmaceutical University Hospital (Osaka, Japan) between January 2016 and December 2024 were included in this study.

This retrospective, single-institution study was conducted in accordance with The Declaration of Helsinki guidelines. The study protocol was approved by the Institutional Review Board of Osaka Medical and Pharmaceutical University Hospital (approval no. 2023-073). All data were anonymised. Informed consent was obtained from the patients using an opt-out methodology, as this retrospective study involved the use of medical records and archived samples with no risk to participants. This study did not include children. Information regarding study inclusion criteria and opt-out options was provided on the institutional website (<https://www.ompu.ac.jp/u-deps/path/img/file19.pdf>).

Cytological analysis. Cervical smear specimens were conventionally stained with Papanicolaou stain. No liquid-based cytology was applied in the present study. Cytological characteristics, including background features (presence of necrotic material or inflammation) and nuclear and cytoplasmic features of neoplastic cells, were evaluated. The cytological features were re-evaluated by at least two researchers in a blind manner. The cytological diagnosis was performed according to the criteria of the Bethesda System for Reporting Cervical Cytology (5). No statistical analysis was performed in the present study.

Histopathological analysis. Surgically resected or biopsied uterine specimens were fixed in 10% buffered neutral formalin, dehydrated, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. The histopathological features of all specimens were independently assessed by at least two researchers. Histopathological features, such as nuclear and cytoplasmic features, were evaluated and compared with the cytological features observed in cervical smear specimens.

Immunohistochemical analysis. Immunohistochemical analysis was performed using an autostainer (Discovery Ultra System; Roche Diagnostics, Switzerland) according to the manufacturer's instructions; 4-micrometre sections were incubated with the following antibodies: rabbit monoclonal antibody against CD56 (MRQ-42, pre-diluted Roche), mouse monoclonal antibody against chromogranin A (LK2H10, pre-diluted, Roche), mouse monoclonal antibody against p16 (E6H4, pre-diluted, Roche), and rabbit monoclonal antibody against synaptophysin (MRQ-40, pre-diluted, Roche). Secondary antibodies were prediluted and incubated at room temperature using the Optiview DAB Universal Kit (cat. no. 518-11427; Roche).

Results

Patient characteristics. Table I summarises the clinicocytological features of the study cohort, which included six female patients (median age, 55 years; range, 39-70 years). Three patients had pure SCNEC, while two had combined SCNEC (in both cases, the other carcinoma component was adenocarcinoma). The remaining patient had carcinosarcoma, in which the carcinoma component was SCNEC, and the sarcomatous component was homologous sarcoma. Patient 6 (carcinosarcoma) received neoadjuvant chemotherapy, followed by radical hysterectomy. Additionally, for Patient 3 (pure SCNEC), only biopsy specimens were available. Human papillomavirus (HPV) testing was not performed on any of the patients.

Cytological features. The characteristic cytological features of cervical SCNEC include the presence of small and/or large clusters of small, round neoplastic cells with round-to-oval nuclei, exhibiting a granular chromatin pattern without conspicuous nucleoli in an inflammatory or necrotic background (Fig. 1A and B). These neoplastic cells also displayed nuclear moulding (Fig. 1B).

In this study, all cytological specimens contained SCNEC components. In patients with pure SCNEC, only SCNEC components were present in the cytological specimens. Among the two patients with combined SCNEC, adenocarcinoma was identified in the cytological specimen of one patient, while the other patient's specimen showed no adenocarcinoma component upon retrospective evaluation. In Patient 1, an adenocarcinoma component was observed, characterised by clusters of columnar cells with peripherally located round-to-oval nuclei and intracytoplasmic mucin (Fig. 1C and D).

For Patient 6 (carcinosarcoma), besides the SCNEC component, small clusters of spindle-shaped cells were observed. These cells had large, oval-to-short spindle nuclei with hyperchromasia and small nucleoli (Fig. 1E and F), suggesting a sarcomatous component.

The initial cytological diagnosis is presented in Table I. According to the Bethesda System for Reporting Cervical Cytology (5), five of the six patients were categorised as having 'other malignant neoplasms (SCNEC)'. The remaining patient (Patient 5) was cytodiagnosed with SCC. Retrospective review revealed that nuclear moulding was present but not apparent in the cytological specimens of Patient 5 compared to the other five patients. The adenocarcinoma component (Patient 1) and the sarcoma component (Patient 6) were not initially detected cytologically. However, in Patient 6, the presence of atypical spindle cells was noted in the cytological report.

Histopathological and immunohistochemical results. Fig. 2 illustrates the typical histopathological features of cervical SCNEC. The proliferation of small, round neoplastic cells with scant cytoplasm and a high nuclear-to-cytoplasmic ratio without conspicuous nucleoli were characteristic of SCNEC (Fig. 2A). These neoplastic cells exhibited nuclear moulding, while mitotic figures and necrosis were also readily observed (Fig. 2B). In patients with combined SCNEC (Patients 1 and 5), an adenocarcinoma component was identified. The adenocarcinoma exhibited glandular formation by neoplastic columnar cells with large, round-to-oval nuclei and prominent

Table I. Clinicocytological features of small neuroendocrine carcinoma of the cervix.

Patient no.	Age, years	Histological features			Cytological features				
		Histology	Other component (%)	Stage	Initial diagnosis	Background	Clusters	Nuclear moulding	Other components
1	59	Combined SCNEC	Adenocarcinoma in situ (10%)	pT1b1	SCNEC	Inflammatory	Small and large	+	Adenocarcinoma
2	41	Pure SCNEC	-	pT1b1	SCNEC	Inflammatory	Small	+	-
3	70	Pure SCNEC	-	NA	SCNEC	Inflammatory	Small and large	+	-
4	39	Pure SCNEC	-	pT1b1	SCNEC	Necrotic	Small and large	+	-
5	52	Combined SCNEC	Adenocarcinoma (30%)	pT1b2	SCC	Necrotic	Small and large	+	-
6	58	Carcinosarcoma	SCNEC and homologous sarcoma components (10%)	ypT1b1	SCNEC	Necrotic	Small and large	+	Sarcoma

NA, not available; SCNEC, small cell neuroendocrine carcinoma; SCC, small cell carcinoma.

NA, not available; SCNEC, small cell neuroendocrine carcinoma; SCC, small cell carcinoma.

nucleoli (Fig. 2C). In Patient 1, no invasive growth was noted (adenocarcinoma *in situ*), whereas infiltrative growth was observed in Patient 5 (Fig. 2C). Proliferation of neoplastic spindle-shaped cells around the SCNEC component was noted in Patient 6 (carcinosarcoma). These spindle-shaped cells had large, oval-to-short spindle nuclei with nucleoli, which were considered to the sarcomatous component (Fig. 2D).

Immunohistochemically, CD56 was diffusely expressed in the SCNEC of all six patients (Fig. 3A). Chromogranin A and synaptophysin were also expressed in the SCNEC component of five patients, except Patient 4 (Fig. 3B and C). Additionally, p16 was diffusely expressed in the SCNEC component of all six patients (100% of SCNEC cells) (Fig. 3D). The adenocarcinoma component did not express CD56, chromogranin A, or synaptophysin; however, p16 was diffusely expressed in the adenocarcinoma components of Patients 1 and 5 (100% of adenocarcinoma cells).

Discussion

This study described the cytological features of six patients with cervical SCNEC. To our knowledge, this is the second cytological series on cervical SCNEC to include combined SCNEC. The cytological features of SCNEC are consistent across various organs, including the lungs, bile duct, uterine cervix, endometrium, and urinary bladder (6-10). Thus, the cytological diagnosis of SCNEC using cervical smears should not be particularly challenging. However, due to the rarity of cervical SCNEC, the rate of accurate cytodagnosis remains low (6,7). For instance, Liu *et al* (7) retrospectively analysed cytological cases of cervical SCNEC, including combined SCNEC. Their study comprised seven pure SCNEC cases and four combined SCNEC, and none of the patients were correctly diagnosed with SCNEC based on the initial cytological examination. Instead, three cases were suspected to have high-grade SIL, while two were misdiagnosed as atypical glandular cells. Additionally, only one cytological specimen contained high-grade SIL, but no adenocarcinoma and SCC components were present by retrospective review of combined SCNEC (7). Cervical SCNEC is mainly missed or misdiagnosed as SIL or SCC (6,7,20-22). In the present series, five of six patients were initially diagnosed with SCNEC, and the remaining patients were diagnosed with SCC. Liu *et al* summarised the causes of misdiagnosis of SCNEC by the cervical cytological examination as follows: i) the rarity of this type of carcinoma, ii) SCNEC can be overlooked in the presence of squamous and/or glandular lesions, iii) SCNEC can be mimicked by the other neoplasms, such as SIL and SCC (cytological differential diagnostic considerations are discussed later), iv) SCNEC is not present in the cytological specimens because this carcinoma component can be present in the submucosal region or necrosis and/or heavy inflammatory may result in insufficient sampling (7). Interestingly, the present series included two patients with combined SCNEC and one patient with carcinosarcoma. Retrospective cytological analysis revealed that the adenocarcinoma component was present in the cytological specimen of one of the two patients with combined SCNEC, and both the sarcomatous component and SCNEC were present in the cytological specimen of the carcinosarcoma. Previous reports have suggested that SCNEC,

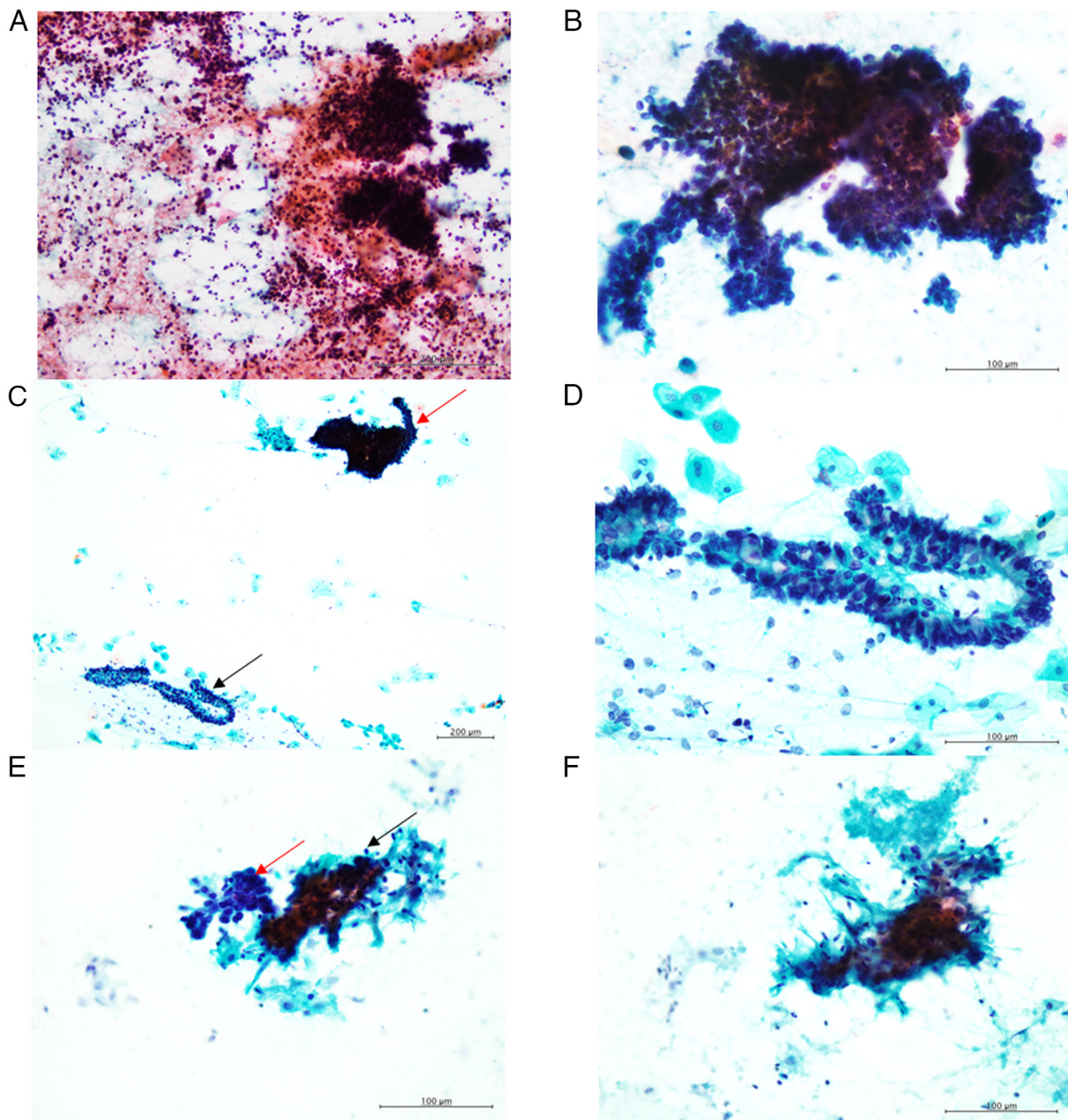


Figure 1. Cytopanels of cervical small cell neuroendocrine carcinoma. (A) Small and large clusters of small, round neoplastic cells in a necrotic background. (B) The neoplastic cells have round-to-oval nuclei containing fine granular chromatin without conspicuous nucleoli and a high nuclear-to-cytoplasmic ratio. Nuclear moulding is a characteristic feature. (C) Both small-cell neuroendocrine carcinoma (red arrow) and adenocarcinoma (black arrow) are present (Patient 1). (D) Adenocarcinoma component consists of columnar neoplastic cells containing large, round-to-oval nuclei and intracytoplasmic mucin. (E) Small cluster of the spindle-shaped neoplastic cells (sarcomatous component) (black arrow) is admixed with small cell neuroendocrine carcinoma (red arrow) (Patient 6). (F) Spindle-shaped neoplastic cells have large oval-to-short spindle nuclei (sarcomatous component) (Patient 6). Papanicolaou staining magnification, (A and C) $\times 100$ and (B and D-F) $\times 400$.

as well as combined adenocarcinoma and/or SIL components, can be detected in cervical cytological specimens (16,17). According to these results, careful observation of cervical cytological specimens may lead to the correct diagnosis of SCNEC as well as combined tumour lesions.

Carcinosarcomas are rare biphasic malignant tumours composed of carcinomatous and sarcomatous components. Although rare, SCNEC can serve as the carcinomatous component in cervical carcinoma (23), as seen in our study (Patient 6). The sarcomatous component is often homologous (e.g., fibrosarcoma, endometrioid stromal sarcoma) but can

also exhibit heterologous differentiation (e.g., osteosarcoma and rhabdomyosarcoma) (23). The accuracy of initial cytological diagnosis for cervical carcinosarcoma is generally low. A previous study reported that only two of 23 cases (21 endometrial and two cervical carcinosarcomas) were correctly identified as carcinosarcoma through cytological examination, while 14 cases were misdiagnosed as carcinoma (24). The detection of sarcomatous components in endometrial and cervical cytological specimens is notably more challenging (24,25). In Patient 6 (carcinosarcoma), the SCNEC component was identified by initial cytological examination.

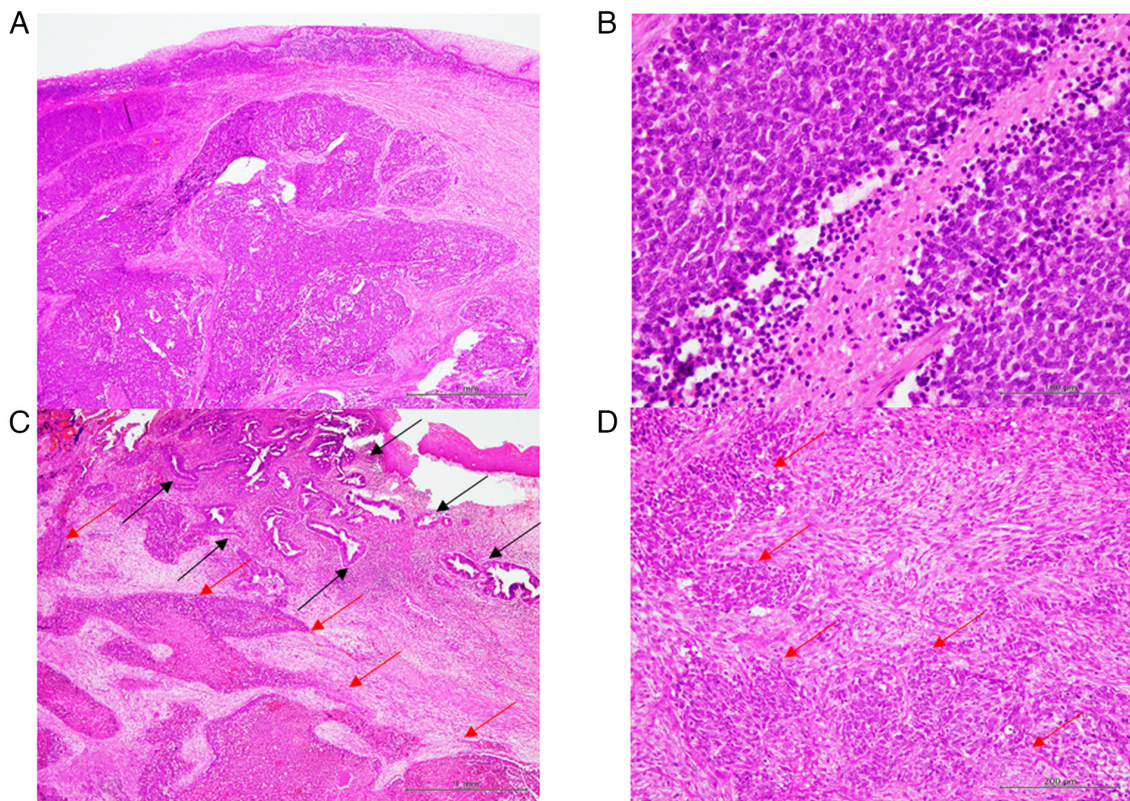


Figure 2. Histopathological features of cervical small cell neuroendocrine carcinoma. (A) Proliferation of small round neoplastic cells is noted under the non-neoplastic cervical squamous epithelium. (B) These neoplastic cells have round-to-oval nuclei without conspicuous nucleoli, and necrosis is also noted within the tumour nest. (C) Combined small cell neuroendocrine carcinoma (Patient 5). Both small cell neuroendocrine carcinoma (red arrows) and adenocarcinoma (black arrows) components are present (D) Proliferation of spindle-shaped neoplastic cells (sarcomatous component) is admixed with small cell neuroendocrine carcinoma component (red arrows) (Patient 6). Haematoxylin and eosin staining magnification, (A and C) x40, (B) x400 and (D) x200.

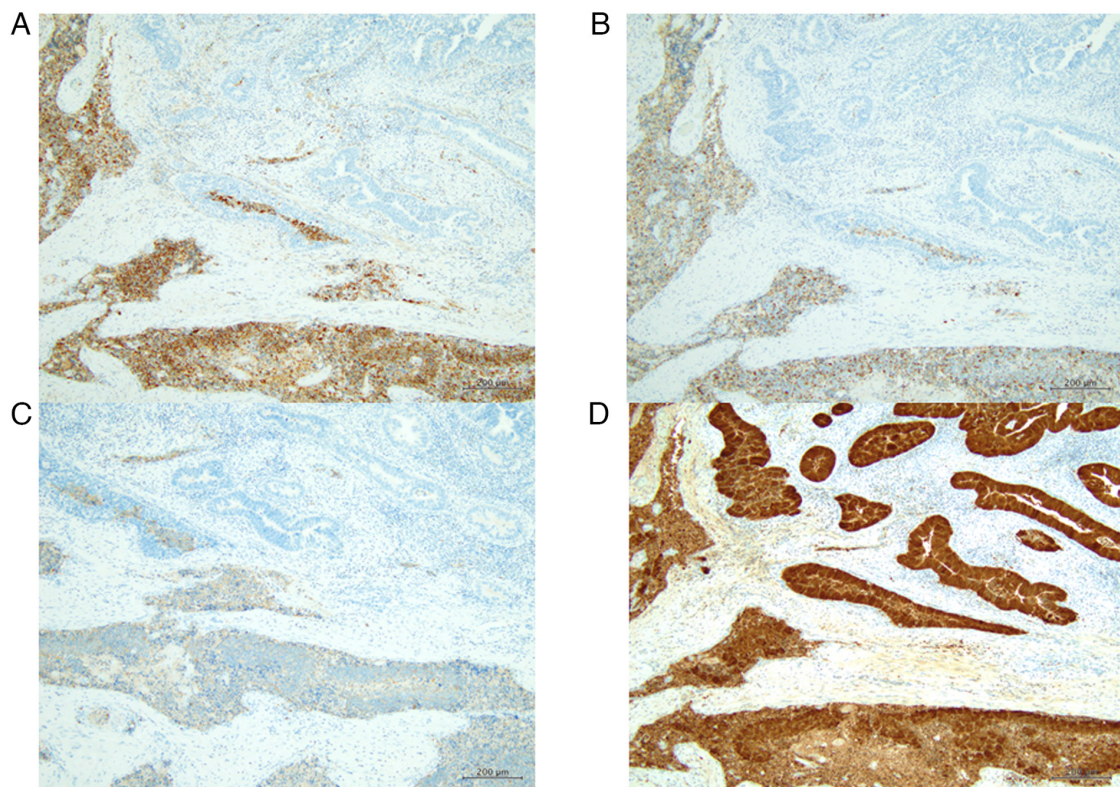


Figure 3. Immunohistochemical features of cervical combined small cell neuroendocrine carcinoma (Patient 5). (A) CD56, (B) chromogranin A and (C) synaptophysin are expressed in the small cell neuroendocrine carcinoma component (left lower) but not in the adenocarcinoma component (right upper). (D) p16 expression is observed in both small cell neuroendocrine carcinoma (left lower) and adenocarcinoma (right upper) components. Magnification, x200.

However, retrospective cytological analysis was able to detect both SCNEC and sarcomatous components. These findings emphasise the importance of meticulous cytological evaluation for achieving an accurate diagnosis.

It is well-recognised that HPV is associated with the development of a large proportion of cervical neoplasms, including SIL, SCC, and adenocarcinoma (5). Infection with HPV, particularly HPV18, a common high-risk strain, is correlated with cervical SCNEC (1,7). Consequently, cervical SCNEC may contain SIL, SCC, and/or adenocarcinoma components, although their precise incidence remains unknown. Immunohistochemical staining for p16 is widely used as a surrogate marker for detecting high-risk HPV, and most cervical SCNECs exhibit positive immunoreactivity for this marker (1,7). Additionally, it has been reported that combined SIL, SCC, and adenocarcinoma components exhibit positive immunoreactivity for p16 (11,16,17). In the present study, all SCNEC showed positive immunoreactivity for p16. Although HPV testing results were not available for all patients in the present cohort, all likely had a high-risk HPV infection, particularly HPV18 (1,7).

The primary differential cytological diagnoses for cervical SCNEC are SIL and SCC. A small proportion of patients with cervical SCNEC are misdiagnosed with SIL and SCC (6,7,20-22), and one of the six patients in the present cohort was initially diagnosed with SCC. Key distinguishing features of SCNEC include finely granular (salt-and-pepper) chromatin, inconspicuous nucleoli, and nuclear moulding (6,7,20-22). In contrast, SIL and SCC typically exhibit richer and denser cytoplasm, clearer cell boundaries, and a lack of definitive nuclear moulding (6,7,20-22). These cytological features may help in differential diagnosis. In one patient initially diagnosed with SCC in the present study, nuclear moulding was not apparent; therefore, SCC was initially considered. Positive immunocytochemical staining for CD56 may aid in the cytodiagnosis of SCNEC (20); however, immunocytochemical staining for neuroendocrine markers was not performed in the present study.

The present study had some limitations. First, this study included relatively few patients with SCNEC, although this type of carcinoma is relatively rare. Second, HPV testing results were not available for all patients in the present cohort. Further studies are needed to clarify the clinicocytological and molecular features of cervical SCNEC.

In conclusion, the results of the present study demonstrated that most SCNEC could be detected in cervical cytological specimens. Moreover, other combined carcinoma components, as well as sarcomatous components, could be identified in cervical cytological specimens. Thus, cervical cytological examination may be helpful in SCNEC diagnosis. The detection of SCNEC in cervical cytological specimens is crucial for accurate and early diagnosis and timely treatment of this aggressive carcinoma.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MO and MI conceived the study. MO, MI, HO, MT, KA, IK, MU, CD, NK, SO, RT, YT, TT, and YH analysed the cytological and/or clinicopathological data, and MO and MI prepared the figures. MO and MI wrote the original draft and edited the manuscript. MO and MI confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was conducted in accordance with the tenets of The Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of Osaka Medical and Pharmaceutical University (approval no. 2023-073; Takatsuki, Osaka, Japan). All data were anonymised. Informed consent was obtained from the patients using an opt-out methodology because of the retrospective study design, with no risk of patient identity exposure. In addition, this study did not include children.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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