Cancer Management and Research

Elevate Series

Open Access Full Text Article

ORIGINAL RESEARCH

Clinicopathological Spectrum and Survival in Esophageal Neuroendocrine Carcinoma and Mixed Neuroendocrine-Non-Neuroendocrine Neoplasms: A 74-Patient Cohort

Lingjuan Zhang[1], Shengli Zhou[2], Zhiquan Gong[2], Man Wu[1], Bingxi Zhou[1], Xiuling Li[1], Jian Li[1], Songze Ding[1]

[1]Department of Gastroenterology and Hepatology, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou, People's Republic of China; [2]Department of Pathology, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou, People's Republic of China

Correspondence: Jian Li; Songze Ding, Email jiuwei@163.com; dingsongze@hotmail.com

Background: Primary esophageal neuroendocrine carcinoma (NEC) and mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs) are rare malignancies with a poor prognosis. Data on clinicopathological features and survival outcomes in Central China are still limited.

Methods: We retrospectively analyzed 74 patients with pathologically confirmed untreated primary esophageal NEC/MiNEN between January 2014 and June 2024. Neuroendocrine and non-neuroendocrine components were evaluated using immunohistochemical staining. The primary outcome was overall survival (OS), which was analyzed using the Kaplan-Meier method and Cox regression.

Results: Among 74 patients with pathologically confirmed primary esophageal NEC/MiNEN, 95.9% had irregular masses with surface erosion or ulcers. 56 patients had small-cell NEC, of whom 16 had adenocarcinoma/squamous cell carcinoma. Eighteen patients had large-cell NEC, including 12 patients with adenocarcinoma/squamous cell carcinoma. Immunohistochemistry showed high positivity for CD56 (98.6%), synaptophysin (Syn, 95.8%), and insulinoma-associated protein 1 (INSM1, 88.5%), but lower for chromogranin A (CgA, 56.9%). Survival analysis showed that there was a significant difference in OS between patients with limited disease (LD, limited to the primary site with regional lymph nodes) and those with extensive disease (ED, with distant metastasis) (58 months vs 9.0 months, $P< 0.001$). The OS of patients with MiNEN was significantly longer than that of patients with pure NEC (76 months vs 23 months, $P< 0.001$), while there was no significant difference in OS between patients with large-cell neuroendocrine carcinoma (LCNEC) and small-cell neuroendocrine carcinoma (SCNEC) ($P= 0.095$). The independent predictors of poor survival were organ metastasis (Hazard Ratio (HR)=3.774, $P=0.003$) and lymph node metastasis (HR=4.569, $P=0.002$).

Conclusion: This study confirms that disease stage (LD vs ED) is one of the most important prognostic determinants of esophageal NEC/MiNEN, and organ and lymph node metastases are independent predictors of poor survival. The findings of this study contribute to improving the predictive efficacy of the prognosis of patients with NEC.

Keywords: esophageal, neuroendocrine carcinoma, MiNEN, immunohistochemistry, treatment, prognosis

Introduction

Neuroendocrine neoplasms (NENs) represent a rare and heterogeneous group of tumors that arise from stem cells and express neuroendocrine markers, enabling them to produce bioactive amines and/or peptide hormones. According to data from the Surveillance, Epidemiology, and End Results (SEER) database of the National Cancer Institute in 2017, the incidence of NENs has increased by approximately 6.4-fold over the past 40 years, outpacing the increase seen in other types of malignant tumors. Among these, the incidence rates for lung and gastroenteropancreatic NENs are 1.49/100,000 and 3.56/100,000, respectively.[1]

According to the World Health Organization (WHO) classification in 2022, digestive tract NENs are categorized into well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs), with the latter requiring distinct therapeutic strategies.[2]

https://doi.org/10.2147/CMAR.S562173
Received: 20 August 2025
Accepted: 1 January 2026
Published: 20 January 2026

At present, there is no standardized treatment for Esophageal NEC.[3] Esophageal NEC constitutes approximately 0.4–2.8% of all esophageal cancers and typically occurs in the middle or lower esophagus. It is a rare but highly malignant tumor that tends to metastasize at an early stage, resulting in poor prognosis.[4–6] In recent years, a number of multi-agency and population-based studies, including SEER-based databases, or analyses that integrate cohorts from several different countries, have provided valuable epidemiological and clinical data for esophageal NEC.[7] Although many studies have analyzed esophageal NEC, owing to the rarity of the disease, most of the existing studies are limited by small sample sizes and lack a comprehensive evaluation of mixed neuroendocrine-non-neuroendocrine tumors (MiNEN) and standardized immunohistochemical classification.

MiNEN represents a biologically and clinically distinct subgroup that has not been adequately described in previous cohorts. Contemporary updates from the WHO and the European Society of Neuroendocrine Tumors (ENETS) emphasize the need for standardized morphological assessments and the application of modern diagnostic teams, including markers such as insulinoma-associated protein 1 (INSM1), synaptophysin, chromogranin A, CD56, TTF-1, p40/p63, and cytokeratin, to more accurately define tumor lineages and identify coexisting non-neuroendocrine components.[2,8] However, few published studies have systematically adopted these updated standards. Although the incidence of esophageal cancer is high in China, especially in central China, a well-annotated cohort that focuses on esophageal NEC and MiNEN is still largely unavailable. Therefore, the prognostic relevance of mixed histology, lymph node involvement, and distant organ metastasis in this population remains unclear.

It is worth noting that there has been no research on esophageal NEC and its related clinical and pathological features in this high-risk area. Therefore, the purpose of this study was to explore the differences in clinical case characteristics and prognosis between pure esophageal NEC and MiNEN, as well as the effects of clinical stage and tumor location on the overall survival (OS) of esophageal NEC. In addition, a panel of biomarkers, including INSM1, chromogranin A (CgA), synaptophysin (Syn), CD56, TTF-1, p40/p63 and Cytokeratin (CK), were systematically evaluated to provide contemporary evidence for accurate diagnosis, risk stratification, and treatment planning of this complex disease group.

Materials and Methods

Demographic Data and Patient Selection

This single-center retrospective cohort study was conducted at Henan Provincial People's Hospital using patients with esophageal cancer who were hospitalized between January 2014 and June 2024. Finally, according to the inclusion/exclusion criteria, 74 patients with esophageal NEC were identified out of 1850 cases of esophageal cancer. Patient details are shown in Table S1. The pathological diagnoses of these patients include pure esophageal or gastroesophageal junction small cell neuroendocrine carcinoma (SCNEC), large cell neuroendocrine carcinoma (LCNEC), and a mixed neuroendocrine-non-neuroendocrine tumor (MiNEN) composed of small cell or large cell neuroendocrine carcinoma and squamous cell carcinoma or adenocarcinoma.

Regional lymph node involvement and distant metastasis were assessed using computed tomography, positron emission tomography-computed tomography, or endoscopic ultrasonography (EUS) examination. If lymph node metastasis could not be definitively determined using these methods, it was confirmed by surgical resection and pathological examination. As this was a retrospective study, the researchers did not adopt a unified treatment plan. After histopathological diagnosis, all the patients were treated according to the clinical practice of our institution. Data on treatment modalities were collected from electronic medical records, including surgical resection, adjuvant therapy, systemic chemotherapy, and palliative care.

The inclusion criteria of patients include: 1. The patients were pathologically diagnosed with primary esophageal or gastroesophageal junction NEC; 2. Tumor types include pure NEC, small-cell NEC, and large-cell NEC. MiNEN: According to the classification of the WHO, it is composed of NEC components (small cells or large cells) and non-neuroendocrine components (such as squamous cell carcinoma or adenocarcinoma), each of which accounts for at least 30% of tumors. 3. Sufficient clinical, pathological, and follow-up data were available for the analysis. The exclusion criteria were as follows: 1. Patients with neuroendocrine tumors that metastasized from non-esophageal primary sites to the esophagus; 2. In patients with neuroendocrine carcinoma, the primary tumor cannot be clearly identified as the

esophagus or gastroesophageal junction; 3. Cases where, despite confirmed pathology, clinical data (age, sex, treatment options) or pathological data (tumor components) were unavailable or incomplete. 4. Cases lacking life status or survival follow-up information; and 5. Patients with well-differentiated neuroendocrine tumors (NETs G1/G2), atypical carcinoids, or other non-cancerous esophageal neuroendocrine tumors.[9,10]

Diagnosis and Classification Criteria

Patients' clinical data, including sex, age, tumor location and size, tumor morphology, clinical stage, and survival outcomes, were collected and analyzed retrospectively. According to the WHO diagnostic criteria for NENs in 2022, the morphological characteristics of esophageal NEC tumor cells under HE staining are consistent with NEC.[2]

The location of esophageal NEC was categorized into three segments based on its distance from the incisors: the upper segment (15–24 cm), the middle segment (25–32 cm), and the lower segment (33–40 cm).[11] According to the Siewert classification for tumors located at the gastroesophageal junction (GEJ), type 1 cancer is defined as a tumor center > 1 cm but less than 5 cm proximal to the GEJ.[12] Type 2 cancer is defined as a tumor epicenter that lies within 1 cm proximal or 2 cm distal to the GEJ. Type 3 cancer is defined as a tumor center > 2 cm but less than 5 cm distal to the GEJ.

The diagnosis of SCNEC is based on its unique histological features: small tumor cells, rare cytoplasm, fine chromatin, and unclear nucleoli. Immunohistochemistry (IHC) showed that SCNEC strongly expressed neuroendocrine markers such as Syn, CgA, and CD56. The definition of LCNEC is: neuroendocrine morphology (organoid nesting, trabecular or rose-like growth pattern, with medium to large tumor cells, prominent nucleoli), and neuroendocrine differentiation is demonstrated by immunohistochemical expression of at least one neuroendocrine marker (Syn, CgA, or CD56) in ≥10% of tumor cells. Pure NEC refers to a tumor composed of only SCNEC or LCNEC components, and does not contain any related non-neuroendocrine epithelial components. When the tumor contains both neuroendocrine carcinoma components (SCNEC or LCNEC) and different non-neuroendocrine carcinoma components (such as adenocarcinoma or squamous cell carcinoma), it is diagnosed as a mixed neuroendocrine-non-neuroendocrine tumor (MiNEN). Each component accounted for at least 30% of the tumor volume.[13]

Considering that there is currently no TNM staging standard for esophageal NEC, it was classified based on the Veterans Administration Lung Study Group (VALG) standard, patients were divided into two groups according to the degree of esophageal NEC.[14] Limited disease (LD) stage refers to tumors limited to the esophagus and its adjacent tissues, with or without regional lymph node metastasis, while extended disease (ED) stage refers to tumors beyond the above range.[15]

The histological subtypes, percentage of MiNEN components, and Ki-67 index were reassessed according to unified WHO (2022) criteria. All staging-related imaging data were reviewed by two radiologists. The differences between the experts reached consensus through consultation. For cases with diagnostic challenges, additional immunohistochemical staining and molecular studies were performed to exclude well-differentiated NETs (such as atypical carcinoids) and confirm the diagnosis.

Immunohistochemistry

To systematically establish the diagnosis and ensure accurate differential diagnosis, a predefined antibody group was applied. Immunohistochemical staining was performed using the BOND-III automatic IHC system (Leica Biosystems, Nussloch, Germany). The following markers were used according to the established diagnostic value, the following markers were used.[16] INSM1 (high sensitivity and nuclear specificity), Syn, CgA, and CD56 (high sensitivity) were used to evaluate neuroendocrine differentiation. Gland and squamous differentiation were assessed by CK/CK8-18 and p40/p63/CK5/6 (diffusely positive), respectively.[17] TTF-1 is incorporated to support small cell morphology, which is positive in most small cell neuroendocrine carcinomas.[18] The primary antibodies used above (Syn, CgA, CD56, INSM1, CK 5/6, p63, TTF-1, p40, CK, CK8/18) were purchased from Abcam (Cambridge, Cambridgeshire, UK). According to WHO classification criteria: SCNEC: cells are small, the cytoplasm is rare, the ratio of nucleus to cytoplasm is high, the chromatin is delicate and "salt-pepper-like," and the nucleolus is not obvious (or missing), often accompanied by nuclear remodeling. LCNEC: cells are large, with medium to rich cytoplasm, rough or vacuolated chromatin, and prominent nucleoli. The Cells are usually arranged in nests or organ-like structures.[19] When > 5–10% of tumor cells are

immunopositive, staining is considered positive.[13,16,20] For descriptive purposes, the positive rate of a single marker is calculated by dividing the number of positive cases by the total number of assessed cases.

IHC staining was performed independently and scored by two pathologists. Neuroendocrine differentiation was confirmed in all cases by IHC detection of at least one of the following markers: Syn, CgA, CD56, or INSM1. In addition, all coexisting components of adenocarcinoma and squamous cell carcinoma were systematically recorded. Only focal neuroendocrine staining (≤5-10%) or isolated single marker-positive tumors are classified as adenocarcinoma or squamous cell carcinoma, with focal neuroendocrine differentiation, thus excluding adenocarcinomas rich in endocrine cells. It is classified as adenocarcinoma or squamous cell carcinoma with focal neuroendocrine differentiation, thus excluding adenocarcinomas rich in endocrine cells.

Follow-up

Patients were followed up either through clinic visits or telephone interviews until August 10, 2024. The overall survival period was calculated in months from the date of pathological diagnosis to the date of death or last follow-up. The minimum follow-up time was half a month and the maximum follow-up time was 107 months. The last contact date was used as the censoring point for patients who were still alive by the deadline.

Statistical Analysis

Data were analyzed using SPSS software (Version 25, IBM Corp, New York, NY, USA). The Mann–Whitney *U*-test was used to compare intergroup differences for continuous variables, and the chi-square test or Fisher's exact test was used for categorical variables, depending on the situation. Multiple comparison data were corrected using the Bonferroni multiple correction. The Kaplan-Meier method was used to generate survival curves, and the median survival time and its 95% confidence interval (CI) were reported. The Log rank test was used to compare differences between the groups. To determine independent prognostic factors, multivariate analysis was performed using Cox proportional hazard regression. To reduce the small sample bias caused by the limited subgroups (such as LCNEC and MiNEN), the Firth penalized likelihood method was used to fit the Cox regression model. Based on the Schoenfeld residual test, the proportional hazard hypothesis of all Cox models was verified and found not to be violated. Statistical significance was set at $P < 0.05$.

Results

Demographic Data of Enrolled Patients

Endoscopic morphological analysis showed that in all 74 patients, 63 (85.1%) were prominent, eight (10.8%) were ulcerative, and three (4.1%) were flat. Among them, The vast majority of prominent and ulcerative lesions (71 cases, 95.9%) were irregular and accompanied by surface erosion. One representative case is shown in Figure 1, which reveals that the lesion extended continuously within the submucosal layer, with part of the lesion connected to the intrinsic muscle layer. The final surgical pathological specimen measured approximately 40×30 ×15 mm. As shown in Table 1, according to the clinical characteristics of the patients, we included 46 patients with pure NEC, including 40 patients with SCNEC and 6 patients with LCNEC. MiNEN patients included 16 patients with mixed NEC and adenocarcinoma (mixed small cell NEC and adenocarcinoma, n=6; mixed large cell NEC and adenocarcinoma, n = 10) and 12 patients with mixed NEC and squamous cell carcinoma (mixed small cell NEC and adenocarcinoma n = 10, mixed large cell NEC and squamous cell carcinoma n = 2).

Clinicopathological Characteristics

The results of H&E staining in patients with LCNEC are shown in Figure 2A. The cells were large in size and arranged in a relatively orderly manner, such as trabeculae, with large nuclei and obvious nucleoli, accompanied by a large amount of necrosis. SCNEC showed that the cells were small, round, oval, with unclear cell boundaries, accompanied by a large number of necrosis and extrusion artifacts (Figure 2B). The demographic data and clinicopathological features of the 74 enrolled patients are summarized in Table 2. There were no significant differences in sex, age, or tumor morphology between the SCNEC and LCNEC groups ($P > 0.05$). There were significant differences in the tumor location, size,

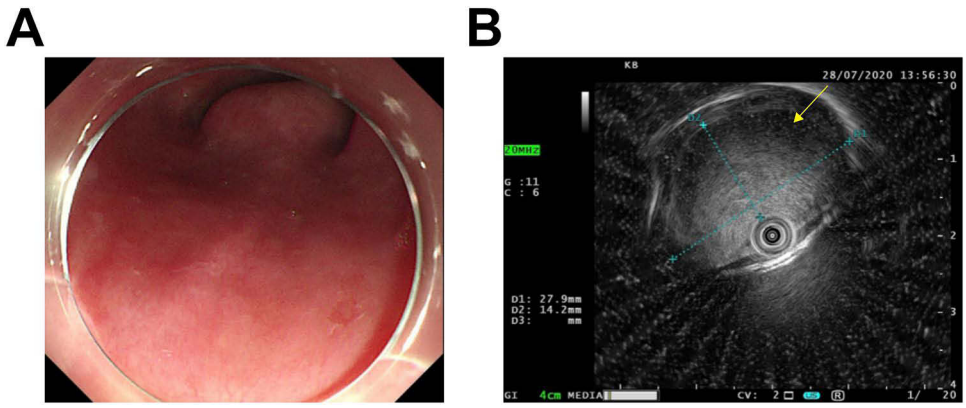

Figure 1 Endoscopic examination results of representative NEC patients. (**A**) Endoscopy showed the morphology of esophageal lesions. (**B**) Endoscopic ultrasonography of esophageal lesions (blue dotted line).
Abbreviation: NEC, Neuroendocrine carcinoma.

clinical stage, and vital statistics ($P < 0.05$). SCNEC tumors were located in the middle esophagus (Mt) (25/ 27), 3/4 in the upper esophagus (Ut), 8/8 in the lower esophagus (Lt), 8/9 between Mt and Lt, and 12/26 at the gastroesophageal junction (GEJ). At the same time, 39/57 of SCNEC were in the LD stage, and 17 cases were all in the ED stage.1 case classified as Siewert type 1,14 case classified as Siewert type 2 and 11 cases as Siewert type 3.

There were 46 patients with pure NEC and 28 patients with MiNEN, including 16 patients with mixed NEC and squamous cell carcinoma (Figure 3A–C) and 12 patients with mixed NEC and adenocarcinoma (Figure 3D and E). HE staining showed that there were two regions: the NEC component region with a high nuclear-cytoplasmic ratio and nuclear shaping, and a squamous cell carcinoma component region with abundant cytoplasm and clear squamous differentiation characteristics (nest-like cells) (Figure 3A). The adenocarcinoma component showed enlarged nuclei, obvious atypia, clear nucleoli, and cells arranged in glandular structures (Figure 3D). The amount of each component exceeded 30% of the total volume of the tumor, indicating that it was MiNEN. There were no significant differences in sex, age, and tumor morphology between pure NEC and MiNEN ($P > 0.05$). In contrast to SCNEC and LCNEC, there was no significant difference in tumor size ($P > 0.05$). There were significant differences in the tumor location, clinical stage, and vital statistics ($P < 0.05$). 31 cases were in the LD stage and 15 cases were in the ED stage in pure NEC, whereas in MiNEN, there were 26 cases in the LD stage and 2 cases in the ED stage. No organ metastasis was observed in the MiNEN group. The metastatic sites of pure NEC patients included the liver (n = 6) and lungs (n = 4). Detailed patient information is provided in Table S1.

Immunohistochemical Staining of NEC Markers

IHC detection was performed using a set of antibody combinations covering neuroendocrine, squamous, and adenoid differentiation markers (Table 3). Positive was defined as more than 30% of tumor cells showing moderate or strong staining.[20] The representative staining results are shown in Figure 3A and B. The neuroendocrine marker INSM1 was positive in the NEC region and negative in adjacent squamous cell carcinoma components (Figure 3B), the squamous

Table 1 Total Number of Cases and Histologic Subtypes

Subtypes	SCNEC	LCNEC	Total
Mixed NEC with adenocarcinoma	6	10	16
Mixed NEC with squamous carcinoma	10	2	12
Pure NEC	40	6	46
Total	56	18	74

Abbreviations: NEC, neuroendocrine carcinoma; SCNC, small cell neuroendocrine carcinoma; LCNC, indicates large cell neuroendocrine carcinoma.

https://doi.org/10.2147/CMAR.S562173

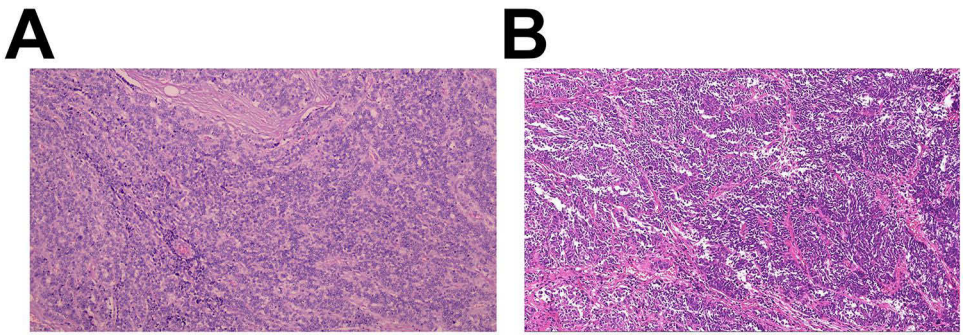

Figure 2 Example of hematoxylin-eosin staining of LCNEC and SCNEC. (**A**) LCNEC (100×). (**B**) SCNEC (100×).
Abbreviations: LCNEC, large cell neuroendocrine carcinoma; SCNEC, small cell neuroendocrine carcinoma.

differentiation marker p40 showed a complementary staining pattern, which was positive in the squamous cell carcinoma region and negative in the NEC region (Figure 3C). Syn was strongly positive in the NEC component, which could be clearly distinguished from the adenocarcinoma region (Figure 3E). In all 74 cases, the positive rates of each marker were as follows: CD56, Syn, CgA, and INSM1 were positive in 98.6% (70/71), 95.8% (69/72), 72.3% (47/65), and 88.5% (23/26), respectively. However, the positive rates of CK and CK8/18 were both 95.1% (39/41), and CK5/6 was at 22.9% (11/48), the positive rates of p63 and p40 were 57.1% (16/28) and 25.5% (12/47), respectively. The positive range of Ki67 in MiNEN

Table 2 Clinicopathological Characteristics of 74 Esophageal NEC

Clinicopathological features	Total	SCNEC (n=56)	LCNEC (n=18)	*P*-value	MiNEN (n=28)	Pure NEC (n=46)	*P*-value
Male	56	40	16	0.24	22	34	0.65
Female	18	16	2		6	12	
Age (mean, years)	65	64	66		67	63	
<60	25	19	6	0.96	7	18	0.21
≥60	49	37	12		21	28	
Tumor location							
Ut	4	3	1	0.00	2	2	0.01
Mt	27	25	2		5	22	
Lt	8	8	0		1	7	
M-Lt	9	8	1		4	5	
GEJ	26	12	14		16	10	
Tumor size (max diameter, cm)							
<4cm	42	37	5	0.00	15	27	0.51
≥4cm	30	17	13		13	17	
Tumor morphology							
Protruded type	63	49	14	0.18	23	40	0.17
Ulcerative type	8	4	4		5	3	
Flat type	3	3	0		0	3	
Clinical stage (VALG)							
LD	57	39	18	0.01	26	31	0.03
ED	17	17	0		2	15	
Vital statistics							
Alive	43	28	15	0.03	22	21	0.01
Dead	31	28	3		6	25	

Abbreviations: NEC, neuroendocrine carcinoma; n, patient number; Ut, upper thoracic esophagus; Mt, middle thoracic esophagus; Lt, lower thoracic esophagus; GEJ, gastroesophageal junction; SCNC, small cell neuroendocrine carcinoma; LCNEC, large cell neuroendocrine carcinoma; VALG, Veterans Administration Lung Study Group; LD, limited disease; ED, extensive disease.

https://doi.org/10.2147/CMAR.S562173

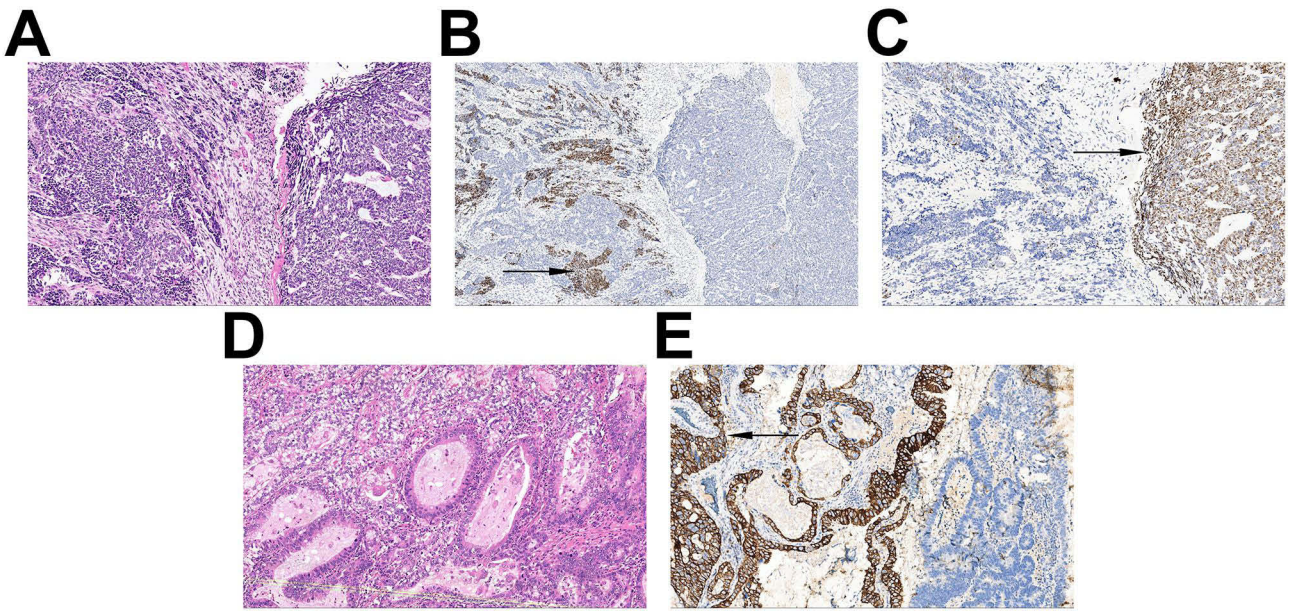

Figure 3 MiNEN immunohistochemical staining example. (**A**) Hematoxylin-eosin stain shows mixed NEC and squamous cell carcinoma (100×). (**B**) IHC shows INSM1 immunopositivity in NEC and immune negativity in the squamous Carcinoma component (as indicated by the arrow, 100×). (**C**) IHC shows p40 immunopositivity in the squamous carcinoma component and immune negativity in NEC (as indicated by the arrow, 100×). (**D**) Hematoxylin-eosin stain shows adenocarcinoma and NEC (100×). (**E**) IHC shows Syn immunopositivity in NEC and immune negativity in the adenocarcinoma component (as indicated by the arrow, 100×).
Abbreviations: Syn, synaptophysin; MiNEN, mixed neuroendocrine–non-neuroendocrine neoplasm; INSM1, insulinoma-associated protein 1; IHC, immunohistochemistry.

was 50% −70%, and the positive range of Ki67 in pure NEC was 70% −80%. Comparing the subtypes of SCNEC and LCNEC, there was no significant difference in the positive rate of immune markers between the two groups ($P > 0.05$), except for the positive rate of Syn ($P = 0.01$). The details of the patient ‘s specific examinations are shown in Table S2.

Prognosis of Patients

A total of 40 patients with LD survived and 17 patients died. The median survival time (MST) was 58 months (95% CI: 29.0–76.0). It is worth noting that the shortest survival time of patients with LD was half a month, and the longest survival time was 107 months. Three total with 3 ED patients survived, and 14 died. The MST was 9 months (95% CI: 4.0–19.0). There was a significant difference in the survival rate between the two groups (log-rank $P < 0.001$) (Figure 4A). Pure NEC

Table 3 Immunostaining for INSM1, CgA, Syn, CD56, CK5/6, p40, p63, CK, CK8/18 and TTF-1

Antigen	Total	Classification		
		SCNEC	LCNEC	*P*-value
INSM1	23/26	16/17	7/9	0.27
CgA	37/65	26/49	11/16	0.39
SYN	69/72	54/54	15/18	0.01
CD56	70/71	54/54	16/17	0.24
CK5/6	11/48	9/39	2/9	1.00
p40	12/47	10/38	2/9	1.00
p63	16/28	15/25	1/3	0.56
CK	39/41	27/29	12/12	1.00
CK8/18	34/36	23/25	11/11	1.00
TTF-1	17/24	15/22	2/2	1.00

Abbreviations: SCNEC, small cell neuroendocrine carcinoma; LCNEC, large cell neuroendocrine carcinoma.

https://doi.org/10.2147/CMAR.S562173

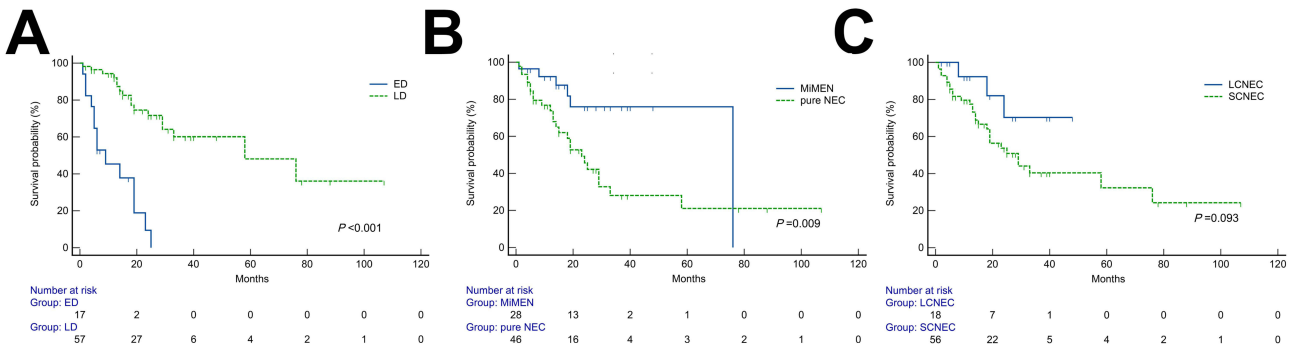

Figure 4 Survival curves of different types of NEC patients. (**A**) Kaplan-Meier survival analysis for LD versus ED. (**B**) Kaplan-Meier survival analysis for pure NEC versus MiNEN. (**C**) Kaplan-Meier survival analysis for SCNEC versus LCNEC.
Abbreviations: ED, extensive disease; LD, limited disease.

patients included 21 surviving and 25 dead patients, with an MST of 23 months (95% CI: 14.0–33.0), including patients with the longest survival time of 107 months. MiNEN patients included 22 patients who survived and died. The MST was 76 months, including patients with a survival time of half month. There was a significant difference in the survival rates between the two groups (log-rank $P = 0.009$) (Figure 4B). SCNEC included 28 patients who survived and 28 who died. The MST was 29 months (95% CI: 18.0–76.0), including the patients with the shortest and longest survival times; LCNEC patients included 15 survivors and 3 dead patients, and the survival rate was higher than 50%. There was no significant difference in the survival rate between the two groups (log-rank $P = 0.093$) (Figure 4C).

Univariate and multivariate analyses were employed to analyze the relationship between clinical and pathological features and prognosis (Table 4). Univariate analysis showed that the prognosis was associated with lymph node metastasis ($P = 0.005$, HR 4.100, 95% CI: 1.544–10.886) and distant organ metastasis ($P = 0.005$, HR=3.246, 95% CI: 1.4–7.4), but there were no

Table 4 Univariate and Multivariate Analysis of Prognostic Factors for Esophageal NEC

Prognostic Parameter	n	Univariate		Multivariate	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age, year					
<60	25	1.0 (reference)			
≥60	49	0.854 (0.402–1.817)	0.682		
Gender					
Male	56	1.0 (reference)			
Female	18	1.102 (0.492–2.467)	0.814		
Tumor size					
<4cm	42	1.0 (reference)			
≥4cm	30	2.109 (0.999–4.450)	0.050		
Lymph node metastasis					
No	27	1.0 (reference)			
Yes	48	4.100 (1.544–10.886)	0.005	4.569 (1.688–12.366)	0.003
Organ metastasis					
No	64	1.0 (reference)			
Yes	10	3.246 (1.432–7.357)	0.005	3.774 (1.634–8.717)	0.002
Chemotherapy					
No	19	1.0 (reference)			
Yes	55	1.524 (0.605–3.842)	0.37		
Treatment method					
Pure resection	16	1.0 (reference)			
Resection plus adjuvant therapy	44	2.314 (0.629–8.516)	0.21		

Abbreviations: NEC, neuroendocrine carcinoma; n, total patient number; HR, hazard ratio; CI, confidence interval.

significant differences in survival time with respect to age ($P = 0.682$, HR=0.854,95% CI: 0.4–1.8), sex ($P = 0.814$, HR=1.102, 95% CI: 0.5–2.5), whether chemotherapy had been received ($P = 0.37$, HR=1.524,95% CI: 0.6–3.8), and whether adjuvant treatment after resection had been received ($P = 0.21$, HR=2.314, 95% CI: 0.6–8.51). In multivariate analysis using Cox proportional-hazards model, the prognosis was significantly associated with lymph node metastasis ($P = 0.003$, HR=4.569, 95% CI: 1.7–12.4) and organ metastasis (P=0.002, HR= 3.774, 95% CI: 1.6–8.7) (Table 4). Lymph node and organ metastases are independent prognostic factors in patients with esophageal NEC.

Discussion

In this work, we summarized the clinical, pathological, and prognostic characteristics of a rare type of esophageal NEC and observed that it exhibits the following features: 1. There were significant differences in tumor location (Ut vs Mt vs Lt vs M-Lt vs GEJ), tumor size (< 4 cm vs ≥ 4 cm), clinical stage (LD vs ED), and vital statistics (alive vs dead) between the SCNEC and LCNEC group; 2. There were significant differences in tumor location, clinical stage, and vital statistics between the MiNEN and pure NEC groups: 3. The overall survival rate of patients with LD was better than that of patients with ED. The prognosis of patients with MiNEN was better than that of patients with pure NEC, and the survival rate of patients with SCNEC was significantly higher than that of patients with LCNEC. 4. Lymph node and distal metastases can be used as independent prognostic factors in patients with esophageal NEC.

The current pathological type of esophageal NEC is mainly SCNEC, and most esophageal NECs are pure NECs. The findings of our study align with those reported by Hong et al in southern China, in their analysis of 80 cases of esophageal and cardia NEC, the majority (76/80) of the patients were classified as small cell type.[11] However, Maru et al reviewed 40 cases of esophageal NEC in the United States and found that 27 out of 40 were large cell types.[21] In contrast, our data indicate that the small cell subtype predominates among NECs in the central China region population. This is an interesting phenomenon that needs to be explored further using larger datasets.

Owing to tumor heterogeneity and limited tissue sampling, preoperative biopsy of esophageal NEN is often poorly correlated with postoperative results. Therefore, postoperative pathology combined with comprehensive immunohistochemical examination is essential for accurate diagnosis.[22,23] To promote standardization and reproducibility, this study was strictly based on the 2022 World Health Organization Classification and ENETS Guidelines. Different from the traditional diagnostic workflow that relies mainly on synaptophysin (Syn) and chromogranin A (CgA),[2] we systematically incorporated INSM1, a highly sensitive and specific marker in gastrointestinal neuroendocrine tumors, and combined with CD56 to enhance the recognition of highly differentiated and poorly differentiated neuroendocrine differentiation.[24,25] Our results confirm the usefulness of this extended panel: the positive rates of INSM1 (88.5%), Syn (95.8%), and CD56 (98.6%) in our cohort are highly consistent with the recent ENETS alignment series.[26] At the same time, MiNEN was effectively identified and poorly differentiated squamous cell carcinoma or adenocarcinoma was excluded by integrating markers such as p40, p63, CK5/6 and CK8/18. Combined with the use of TTF-1, we further speculated on its potential source. This multi-label method provides a more rigorous diagnostic benchmark than simple morphology, reduces the variability between centers, and ensures accurate subclassification.

In our cohort, the prognosis of MiNEN patients (37.8%, 28/74 cases) was significantly better than that of pure NEC patients. The MST of pure NEC was 23 months and that of MiNEN was 76 months. Previous studies have also suggested that the MST of MiNEN is longer than that of pure NEC.[11,21] This is consistent with our research trend. This may be related to the early performance. The vast majority of MiNEN patients (22/28 cases) were in the LD stage, and there was no distant metastasis. This suggests that the histological subtype itself may not be an independent prognostic factor, but together with a more favorable disease stage, constitutes the basis for a good prognosis of MiNEN. It is worth noting that some MiNENs showed complex epithelial heterogeneity, including one case with three different components (NEC, squamous cell carcinoma, and adenocarcinoma), and another case of SCNEC coexisting with adjacent early squamous cell carcinoma. These phenomena, together with the expression of the stem cell marker p63 in non-neuroendocrine components and the loss of CK20, support that esophageal neuroendocrine carcinoma may originate from endothelial-derived pluripotent stem cells and retain the potential to differentiate into different epithelia after malignant transformation.[27,28] These findings underscore the importance of adequate sampling and comprehensive immunohistochemistry, especially in biopsy specimens, to identify mixed components that may influence treatment options.

This study found that although the OS between LCNEC and SCNEC did not reach statistical significance, LCNEC showed a better prognostic trend than SCNEC, suggesting that SCNEC may be more invasive. This observation is consistent with the conclusion of Tustumi et al, that is, the prognosis of esophageal LCNEC is better than that of SCNEC.[29] Similarly, Mao et al compared the clinical characteristics and prognosis of lung LCNEC with small cell lung cancer (SCLC) and found that the survival advantage of LCNEC was significant in patients with stage I–II, but not in patients with stage III.[30] In this study, the overall survival of LD patients was significantly better than that of ED patients. It is worth noting that all LCNEC cases were LD, whereas the SCNEC group included both LD and ED patients, and its survival time distribution was extremely scattered, from short-term survival (0.5–6 months) to long-term survival (88 and 107 months). This significant stage heterogeneity is likely to affect the direct survival comparison between SCNEC and LCNEC patients. Patients with LD had a better survival outcome than those with ED, with an MST of 33 and 6 months, respectively. This finding is in line with previous studies, underscoring the critical role of early diagnosis in enhancing the prognosis of esophageal NEC.[21,31] This inference still needs to be verified in the future through a larger sample size and more detailed staging and subtype stratification studies.

In this study, organ and lymph node metastases were identified as independent predictors of prognosis. Among all cases, 48 developed lymph node metastases, while 10 developed distant organ metastasis. We observed that ED, SCNEC, and distant metastasis had poor prognoses. This group of high-risk patients is the most urgent clinical need to go beyond traditional chemotherapy and more active multimode comprehensive treatment candidates. In the treatment of NEC and MiNEN, the common treatment strategy is surgery combined with adjuvant chemotherapy.[32] Sometimes immunotherapy or other methods are sometimes required. Zhang et al treated a 45-year-old male patient with oligometastatic stage IV LCNEC with chemotherapy and serplulimab (a programmed cell death protein 1 inhibitor) during the perioperative period. After 17 cycles of treatment, it was clinical cure.[33] In the treatment of a patient with primary small cell neuroendocrine carcinoma of the ureter, Li et al used neoadjuvant etoposide plus carboplatin chemotherapy, followed by resection surgery, and assisted with PD-1 inhibitor combination therapy. The patient ‘s tumor was significantly inhibited, and no recurrence occurred during the 12-month follow-up period.[34] This suggests that platinum combined with immunosuppressive therapy may be an effective surgical treatment strategy for this high-grade neuroendocrine carcinoma. However, owing to individual heterogeneity during treatment, there are no standard treatment guidelines, and treatment strategies may need to be continuously adjusted. Some patients may be less sensitive to immunosuppressive therapy, which requires continuous exploration of clinical trials of new therapies such as combined immune checkpoint inhibitors. Therefore, future prospective studies should be devoted to verifying the efficacy of combined therapy in the above-mentioned high-risk groups and simultaneously explore predictive biomarkers such as PD-L1 and MSI/tumor mutation burden (TMB) to achieve true precision treatment.

Although this study provides valuable information related to the diagnosis, treatment, and prognosis of esophageal NEC, it has some limitations. First, there was no analysis of the differences in treatment methods and prognoses of different subgroups. In the future, more samples need to be collected, and cell and animal experiments need to be further verified. Second, the limited sample size in certain subgroups reduced the reliability of the comparative analyses, and some statistical differences did not achieve statistical power. Third, as this study was a single-center retrospective analysis, its findings may reflect only the characteristics of the disease within the local region, and the results and conclusions may not apply to other regions. Fourth, specific treatment details (such as the use of chemotherapy cycles, radiotherapy regimens, or immunotherapy) and missing data on some clinicopathological variables hindered a more detailed analysis. Fifth, this study did not include smoking, drinking, and other lifestyle statistics; these potential factors may be associated with the disease and the site of impact. Sixth, this study did not explore the prognostic value of Ki67 in this cohort.

To ensure the rigor and accuracy of scientific research, we anticipate that the results of this study should be further validated through large-scale, well-designed, multicenter, prospective studies, which will be helpful for clear survival analysis and the development of evidence-based standardized treatment algorithms. Second, there is an urgent need for comprehensive molecular analysis of these tumors, including assessment of changes in key drivers (such as RB1 and TP53), mismatch repair status/MSI, TMB, and potential therapeutic targets. This analysis can identify biologically distinct subgroups, explain the observed clinical heterogeneity, and guide the application of targeted therapy or immunotherapy. To design prospective studies to systematically evaluate the potential benefits of neoadjuvant therapy in patients with different molecular subtypes or risk stratifications, a standardized comprehensive management pathway for esophageal cancer was constructed, and perioperative

treatment strategies (including neoadjuvant therapy) were included in the quantifiable evaluation. However, despite these limitations, the current study has provided important information for this rare type of tumor in this region, which deserves further exploration to optimize treatment strategies and improve patient outcomes.

Conclusion

In summary, this retrospective study of regional cohorts revealed the clinicopathological features of primary esophageal neuroendocrine carcinoma (NEC). Under the limitations of our sample size and design, the key data-driven findings are as follows: (1) there was a significant difference in survival rate between patients in the LD and ED stages, and there was a significant difference in survival between pure NEC and MiNEN patients. There was no significant difference in survival rates between SCNEC and LCNEC. Thus, the prognosis of patients with LD and MiNEN may be better. (2) Multivariate analysis identified the presence of lymph node metastasis and distant organ metastasis as independent predictors of low survival rates, emphasizing their key role in risk stratification. This observation warrants further verification in a larger study. These observations underscore the need for precise histological sub-classification and comprehensive staging to guide the treatment of this deadly disease. These elements should be the cornerstone of future multi-agency efforts to develop robust prognostic models and standardized treatment algorithms. Exploratory observations of potential clinical heterogeneity (eg, the behavior of MiNEN and pure NEC) and treatment patterns can be used as hypotheses for subsequent studies that combine prospective clinical data with molecular analysis to identify predictive biomarkers and therapeutic targets.

Data Sharing Statement

All the results are presented in the article. Further inquiries can be directed to the corresponding author Songze Ding.

Ethics Statement

The research protocol was approved by the Ethics Committee of Henan Provincial People's Hospital (No.114, 2022). All experiments and procedures were performed according to the Declaration of Helsinki (as revised in 2013).

Funding

This work is supported by the National Natural Science Foundation of China (NO U1604174) and Health Commission of Henan Province-Province and Ministry Co-construction Project (SBGJ202002004).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Dasari A, Shen C, Halperin D, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. *JAMA Oncol*. 2017;3(10):1335–1342. doi:10.1001/jamaoncol.2017.0589
2. Rindi G, Mete O, Uccella S, et al. Overview of the 2022 WHO classification of neuroendocrine neoplasms. *Endocrin Pathol*. 2022;33(1):115–154. doi:10.1007/s12022-022-09708-2
3. Pourfaraji SM, Jalaeefar A, Ojaghi Shirmard F, Salabat D, Mohammadi S, Mohammadzadeh N. Neuroendocrine carcinoma of esophagus: systematic review and meta-analysis of case series. *BMC Gastroenterol*. 2025;25(1):462. doi:10.1186/s12876-025-04078-5
4. Xu L, Li Y, Liu X, et al. Treatment strategies and prognostic factors of limited-stage primary small cell carcinoma of the esophagus. *J Thorac Oncol*. 2017;12(12):1834–1844. doi:10.1016/j.jtho.2017.09.1966
5. Ji A, Jin R, Zhang R, Li H. Primary small cell carcinoma of the esophagus: progression in the last decade. *Ann Translat Med*. 2020;8(7):502. doi:10.21037/atm.2020.03.214
6. Jeene PM, Geijsen ED, Muijs CT, et al. Small cell carcinoma of the esophagus: a Nationwide Analysis of Treatment and Outcome at Patient Level in Locoregional Disease. *Am J Clin Oncol*. 2019;42(6):534–538. doi:10.1097/COC.0000000000000546
7. Lin Z, Chen YY, Liu CH, et al. Comparison of clinicopathological features and survival analysis between esophageal neuroendocrine carcinoma and esophageal squamous cell carcinoma based on the SEER database, alongside nomogram analysis for esophageal neuroendocrine carcinoma. *J Gastrointest Oncol*. 2023;14(6):2309–2323. doi:10.21037/jgo-23-905
8. Panzuto F, Ramage J, Pritchard DM, et al. European Neuroendocrine Tumor Society (ENETS) 2023 guidance paper for gastroduodenal neuroendocrine tumours (NETs) G1-G3. *J Neuroendocrinol*. 2023;35(8):e13306. doi:10.1111/jne.13306
9. Hong Q, Wu K, Chen C, et al. Characterizing esophageal mixed neuroendocrine-non-neuroendocrine neoplasms: insights from a retrospective multicenter study of clinical outcomes and prognostic indicators. *Therapeut Adv Med Oncol*. 2024;16:17588359241303066. doi:10.1177/17588359241303066

10. Wu IC, Chu YY, Wang YK, et al. Clinicopathological features and outcome of esophageal neuroendocrine tumor: a retrospective multicenter survey by the digestive endoscopy society of Taiwan. *J Formosan Med Assoc*. 2021;120(1 Pt 2):508–514. doi:10.1016/j.jfma.2020.06.024
11. Hong L, Zhang Y, Liu Z. Neuroendocrine carcinoma of esophageal and gastric cardia: clinicopathologic and immunohistochemistry study of 80 cases. *Oncotarget*. 2018;9(12):10754–10764. doi:10.18632/oncotarget.23610
12. Siewert JR, Stein HJ. Classification of adenocarcinoma of the oesophagogastric junction. *Br J Surg*. 1998;85(11):1457–1459. doi:10.1046/j.1365-2168.1998.00940.x
13. Gao R, Zhang X, Chen X, et al. Comparison of insulinoma-associated protein 1 (INSM1) with traditional neuroendocrine markers in gastrointestinal and pancreatic mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs). *Diagn Pathol*. 2024;19(1):144. doi:10.1186/s13000-024-01568-0
14. Micke P, Faldum A, Metz T, et al. Staging small cell lung cancer: Veterans Administration Lung Study Group versus International Association for the Study of Lung Cancer--what limits limited disease? *Lung Cancer*. 2002;37(3):271–276. doi:10.1016/S0169-5002(02)00072-7
15. Van Der Gaast A, Verwey J, Prins E, Splinter TA. Chemotherapy as treatment of choice in extrapulmonary undifferentiated small cell carcinomas. *Cancer*. 1990;65(3):422–424. doi:10.1002/1097-0142(19900201)65:3<422::AID-CNCR2820650308>3.0.CO;2-Y
16. Huang R, Yu L, Zheng C, et al. Diagnostic value of four neuroendocrine markers in small cell neuroendocrine carcinomas of the cervix: a meta-analysis. *Sci Rep*. 2020;10(1):14975. doi:10.1038/s41598-020-72055-x
17. Sun H, Shi Y, Liang D, Liu J, Xu G, Yang X. Esophageal mixed or collision small cell-squamous cell carcinoma: report of 6 cases and review of the literature. *Indian Journal of Cancer*. 2024;61(4):759–765. doi:10.4103/ijc.IJC_173_21
18. Recuero E, Lázaro S, Lorz C, Enguita AB, Garcia-Escudero R, Santos M. Novel mouse cell lines and in vivo models for human high-grade neuroendocrine lung carcinoma, Small Cell Lung Carcinoma (SCLC), and Large Cell Neuroendocrine Carcinoma (LCNEC). *Int J Mol Sci*. 2023;24(20):15284. doi:10.3390/ijms242015284
19. La Rosa S. Diagnostic, prognostic, and predictive role of Ki67 proliferative index in neuroendocrine and endocrine neoplasms: past, present, and future. *Endocrin Pathol*. 2023;34(1):79–97. doi:10.1007/s12022-023-09755-3
20. Fujita Y, Uesugi N, Sugimoto R, Eizuka M, Matsumoto T, Sugai T. Gastric mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) with pancreatic acinar differentiation: a case report. *Diagn Pathol*. 2019;14(1):38. doi:10.1186/s13000-019-0815-3
21. Maru DM, Khurana H, Rashid A, et al. Retrospective study of clinicopathologic features and prognosis of high-grade neuroendocrine carcinoma of the esophagus. *Am J Surg Pathol*. 2008;32(9):1404–1411. doi:10.1097/PAS.0b013e31816bf41f
22. Jepsen DNM, Fiehn AK, Garbyal RS, Engel U, Holm J, Federspiel B. Immunohistochemical staining with neuroendocrine markers is essential in the diagnosis of neuroendocrine neoplasms of the esophagogastric junction. *AIMM*. 2021;29(6):454–461. doi:10.1097/PAI.0000000000000906
23. Sulaiman M, Agarwal S, Mandal A, Kumar S, Das P. High-grade mixed neuroendocrine non-neuroendocrine neoplasm of the gastroesophageal junction: a rare case report and review of literature. *Indian J Pathol Microbiol*. 2022;65(4):918–920. doi:10.4103/ijpm.ijpm_143_21
24. Wang XQ, Fu J, Zhang YT, Xu Y. INSM1 expression in primary and metastatic neuroendocrine neoplasms at distinct locations. *Pathol Res Pract*. 2024;253:155067. doi:10.1016/j.prp.2023.155067
25. McHugh KE, Mukhopadhyay S, Doxtader EE, Lanigan C, Allende DS. INSM1 is a highly specific marker of neuroendocrine differentiation in primary neoplasms of the gastrointestinal tract, appendix, and pancreas. *Am J Clin Pathol*. 2020;153(6):811–820. doi:10.1093/ajcp/aqaa014
26. Sorbye H, Grande E, Pavel M, et al. European Neuroendocrine Tumor Society (ENETS) 2023 guidance paper for digestive neuroendocrine carcinoma. *J Neuroendocrinol*. 2023;35(3):e13249. doi:10.1111/jne.13249
27. Walenkamp AM, Sonke GS, Sleijfer DT. Clinical and therapeutic aspects of extrapulmonary small cell carcinoma. *Cancer Treat Rev*. 2009;35(3):228–236. doi:10.1016/j.ctrv.2008.10.007
28. Sun KL, He J, Cheng GY, Chai LX. Management of primary small cell carcinoma of the esophagus. *Chin Med J*. 2007;120(5):355–358. doi:10.1097/00029330-200703010-00001
29. Tustumi F, Marques SSB, Barros EF, Henriques AC, Waisberg J, Dias AR. The prognosis of the different esophageal neuroendocrine carcinoma subtypes: a population-based study. *Arquivos de gastroenterologia*. 2022;59(1):53–57. doi:10.1590/s0004-2803.202200001-10
30. Mao Y, Chang H, Wang L, et al. Comparison of clinicopathologic features and prognosis between surgically resected pulmonary large-cell neuroendocrine carcinoma and small-cell lung cancer. *J Thoracic Dis*. 2025;17(4):2394–2410. doi:10.21037/jtd-2025-345
31. Zhang X, Ma L, Bao H, Zhang J, Wang Z, Gong P. Clinical, pathological and prognostic characteristics of gastroenteropancreatic neuroendocrine neoplasms in China: a retrospective study. *BMC Endocr Disord*. 2014;14:54. doi:10.1186/1472-6823-14-54
32. Zhang S, Zheng C, Chen Y, et al. Clinicopathologic features, surgical treatments, and outcomes of small bowel tumors: a retrospective study in China. *Int J Surg*. 2017;43:145–154. doi:10.1016/j.ijsu.2017.05.076
33. Zhang S, Du J, Lin C, et al. Perioperative serplulimab-based chemoimmunotherapy in stage IV large cell neuroendocrine carcinoma of the lung: a case report. *Oncol Lett*. 2024;28(5):547. doi:10.3892/ol.2024.14680
34. Li Y, Deng Y, Ma S, Liu J. Multimodal therapy for primary ureteral small cell neuroendocrine carcinoma with high-grade urothelial component: case report and literature review. *Front Urol*. 2025;5:1615270. doi:10.3389/fruro.2025.1615270