

Clinical characteristics and prognostic analysis of gastroenteropancreatic neuroendocrine neoplasm (GEP-NEN)

Ping Yang¹, Jie Liu², Dongliang Lin¹, Haiyang Fu¹, Jing Chu¹, Feng Li¹, Guiyan Han¹, Yujun Li (✉)¹, Weiwei Fu (✉)¹

¹ Department of Pathology, The Affiliated Hospital, Qingdao University, Qingdao 266003, China

² Digestion Department of Internal Medicine Hospital Economic and Technological Development Zone, Yantai 264000, China

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Abstract *Objective:* The aim of the study was to analyze the clinicopathologic characteristics of gastroenteropancreatic neuroendocrine neoplasm (GEP-NEN) and to explore the prognostic factors for patients and differences of immunohistochemical markers between neuroendocrine tumor (NET) and neuroendocrine carcinoma (NEC). *Methods:* Retrospective reviews were conducted for the charts of 119 patients with GEP-NEN at the Affiliated Hospital of Qingdao University (China) from August 2003 to December 2013. Kaplan-Meier method was used to do the overall survivals analysis for the patients at different levels of predictive factors. Meanwhile, Cox proportional hazard model was used to select independent risk factors of survival. Analysis of variance was used to compare the expression of immunohistochemical markers among different pathological grades. *Results:* Among 119 patients, pancreas (45/119, 37.82%) and rectum (33/119, 27.73%) were mostly involved. The onset age of GEP-NEN in female group was younger than that of the male group. There were 13 deaths (10.92%) during 18.9 (0.1–133.4) months follow-up period. Multivariate analysis indicated that neural invasion, gender and pathological grades of NET and NEC were independent risk factors. In neuroendocrine neoplasm (NEN), Syn expression in G2 was higher than G1 and G3, while CgA showed no significant difference. All markers showed no significant differences between NET and NEC. *Conclusion:* GEP-NEN may occur at multiple sites of digestive system and lack specific clinical manifestations. Syn expression detected for the prognosis of G1, G2 and G3 tumors have clinical significance. Neural invasion, sex and pathological grades were independent prognostic factors for GEP-NEN patients. No significant difference was found in different pathological grades of NET and NEC.

Key words neuroendocrine neoplasm (NEN); pathology, prognosis; multiple factor analysis

Neuroendocrine neoplasm (NEN) is a kind of heterogeneous tumor which is originated from peptidergic neurons and neuroendocrine cells pervading in the neuroendocrine system. The NEN could secrete a variety of active hormone and differentiate in multi-directions. NEN can occur in many organs and tissues. The gastrointestinal tract and the pancreas are the most common sites with the percentage of 70% [1]. The morbidity of gastroenteropancreatic neuroendocrine neoplasm (GEP-NEN) is low, but the growth of morbidity is increasing – about 50/100 thousand, which was ranked the second place of the digestive tract cancers [2]. Therefore, it is of clinical significance to further analyze the pathological

characteristics of GEP-NEN. This retrospective research reviewed the pathological and follow-up data of 119 GEP-NEN postoperative large specimens of the Affiliated Hospital of Qingdao University (China) from 2003 to 2013. In this study, we investigated the clinical pathological features and prognostic factors of those cases to further understanding of the pathogenesis of this disease.

Materials and methods

Materials

The 119 cases of GEP-NEN were recruited from the Affiliated Hospital of Qingdao University (China) from August 2003 to December 2013. The chromogranin A (CgA), synaptophysin (Syn) and Ki-67 used for diagnosis were bought from Beijing Zhongshan Biotechnology

Correspondence to: Yujun Li. Email: liyujun.66@163.com
Weiwei Fu. Email: eer-df@163.com

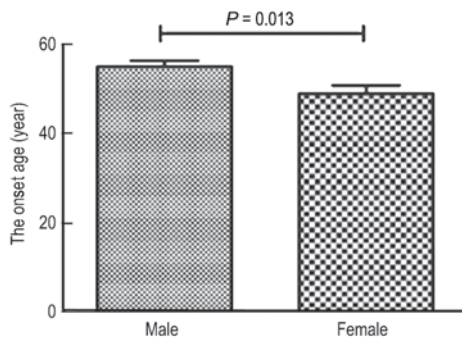


Fig. 1 The difference of onset ages between male and female

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Methods

Specimens were fixed in 4% neutral formalin, then under the process of conventional dehydration and paraffin embedding. After that, they were cut into 4 μm thick slices with HE staining to observe the morphological characteristics. Immunohistochemical SP method was used to detect Ki-67, CgA, Syn expressions in tumor tissues. We set negative and positive controls and strictly followed the instructions of product manual. And CgA, together with Syn in tumor cell cytoplasm in tan particle shades was positive. Tumor nuclear fission and Ki-67 index were conducted based on China gastrointestinal pancreatic neuroendocrine tumor pathology diagnosis consensus [3]. The outcome was blindly checked and analyzed by two pathologists. All the cases were classified according to the WHO standards for GEP-NEN and grouped into G1, G2 and G3. For the differentiation of tumor cells, they were grouped into neuroendocrine tumor (NET), neuroendocrine carcinoma (NEC), mixed adenoendocrine carcinoma (MANEC) and NEN [4].

Statistic methods

This research conducted follow-up visit via phones or police station. And the data were analyzed by SPSS 13.0 statistical software. Kaplan-Meier method and Log-rank method were used respectively for survival analysis and survival analysis inspection. Cox regression analysis was conducted to analyze risk factors for survival. Measurement data were used χ² test. *P* < 0.05 was considered significant.

Results

Analysis of clinical pathological sources

The study subjects consisted of 72 males and 47 females, with an average age of 56.2 years (19–86 years). The onset ages of male and female were statistical significant (*P* = 0.013). As shown in Fig. 1. The average tumor size (maxi-

Table 1 CgA expression in different organs

Parts	Total	CgA positive expression	Percentage
Pancreas	45	45	100
Rectum	33	1	3.03
Gastric body	15	12	80
Distal	11	10	90.91
Colon	6	0	0
Ileocecum	3	0	0
Stomach Angle	3	2	66.67
Stomach bottom	2	2	100
Cardiac	1	1	100

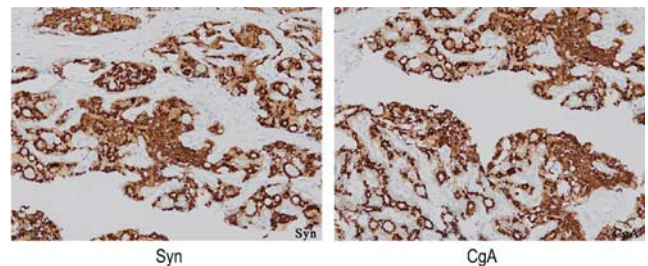


Fig. 2 The immunohistochemistry positive expressions of Syn and CgA (Positive staining × 100)

um diameter) was 2.72 cm (0.3 to 11 cm). There were 76 cases in G1 (63.9%), 24 cases in G2 (20.2%) and 19 cases in G3 (15.9%), including 4 cases of large cell NEC, 3 cases of small cell NEC and 12 cases of MANEC.

Among 119 cases, there were 45 cases (37.82%) occurred in pancreas, 33 cases in rectum (27.73%), 26 cases in gastric body and the gastric antrum (21.85%), 6 cases in colon (5.04%), 3 cases (2.52%) in the small intestine, 3 cases in angle of the stomach, 2 cases in fundus (1.68%), and 1 case in cardia (0.84%). GEP-NEN patients usually occurred in the pancreas and rectum (47 males, 65.28%; 31 females, 65.96%). Of the 119 patients with GEP-NEN, 32 cases (26.89%) were functional, 87 cases (73.11%) without function. Functional tumor all occurred in the pancreas, including 30 cases of insuloma and 2 cases of growth inhibition of melanoma. Nonfunctional tumor mainly presented as abdominal discomfort, dark stool, bloody stool, diarrhea, changes in bowel habits, weight loss and other non-specific gastrointestinal symptoms.

Outcome of immunohistochemical staining

Among 119 cases of tumor specimens, 73 cases (61.34%) specimens were CgA positive, 119 cases (100%) were Syn positive. CgA expression in different parts of the tumor was significantly different (Table 1 and Fig. 2).

But for CgA expression there were no significant differences between NET and NEC (*P* = 0.466) just as in different pathological grades (*P* = 0.399, *P* = 0.466). In Fig. 2, Syn expression in G1, G2 and G3 was statistically signifi-

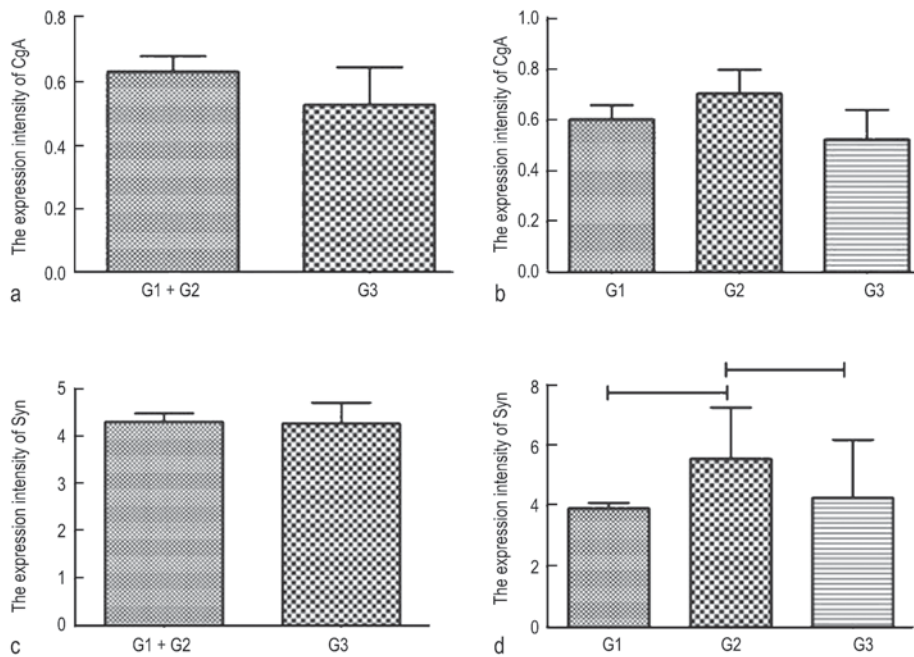


Fig. 3 CgA and Syn expressions in different pathological grades. (a and b) For CgA expression there were no significant differences between NET and NEC ($P = 0.466$) just as in different pathological grades ($P = 0.399$, $P = 0.466$); (c) No significant differences were in the expression of NET (G1 + G2) and NEC (G3) ($P = 0.599$); (d) Syn expression in G1, G2 and G3 was statistically significant ($P < 0.001$)

cant ($P < 0.001$), the expression of Syn in G2 was higher than in G1 and G3, whereas no significant difference was observed between G1 and G3 ($P = 0.066$). However, no significant differences were in the expression of neuroendocrine tumors (G1 + G2) and neuroendocrine carcinomas (G3) ($P = 0.599$; Fig. 3).

Survival analysis

The follow-up time of 119 cases was ranging from 0.1 to 133.4 months with the median follow-up time of 18.9 months. Thirteen cases (10.92%) died. The 1-, 3-, and 5-year survival rates were 96.61%, 91.61% and 90.76% respectively. Of the 119 patients 81 cases were under 60 years (68.06%), 9 cases (11.11%) died. And 38 cases were over 60 years, 4 cases (10.53%) of which died. Overall survival among different ages showed no significant difference ($P = 0.575$), as shown in Fig. 4.

In 72 male patients, 12 cases died (16.67%) while 1 case died in 47 female patients (2.13%). Overall survival between different gender was statistically significant ($P = 0.016$), as shown in Fig. 4.

In all patients, 14 cases were vascular invasion positive

(11.76%), 5 cases died (35.71%), 105 cases were vascular invasion negative (88.24%), 8 cases died (6.72%), overall survival between different vascular invasion groups with statistical difference ($P = 0.005$; Fig. 4).

All of the patients, 14 cases were nerve invasion positive (11.76%), 6 cases died (42.86%), and 105 cases were nerve invasion negative (88.24%), 7 cases died (6.67%), different neural invasion overall survival between groups had statistically significant differences ($P < 0.001$).

As shown in Fig. 4, in lymph node, 17 cases were metastasis positive (14.29%), 7 cases died (41.18%), with no lymph node metastasis of 102 cases (85.71%), 6 cases died (5.88%), overall survival difference between different lymph node metastasis groups were statistically significant ($P < 0.001$). Different histological level, respectively, G1: 2 death (2.63%), G2: 3 deaths (12.5%), and G3 (here the NTC and MANEC in G3 as statistical prognosis): 8 deaths (42.11%). Overall survival between different grading had significant statistical significance ($P < 0.001$).

In immunohistochemical results, 8 cases were Syn weakly positive, 34 cases were moderately positive and 77 cases were strongly positive, and the survival time was no significant difference among different expression intensities ($P = 0.95$); 46 cases were CgA negative, 73 cases were positive, survival time among different expression intensities had no significant difference ($P = 0.85$), as shown in Fig. 4.

The prognosis of GEP-NEN patients of multi-factor analysis indicated that the grade of tumor, the patients with nerve invasion and gender were independent risk factors (survival time). Male patient, patients with nerve

Table 2 Multi-factor analysis of Cox

Variables	HR (95% CI)	P
Classification of tumor		
G1 + G2 / G3	4.55 (1.67-12.42)	0.003
Nerve infiltration		
Y / N	4.23 (1.04-17.20)	0.044
Sex		
M / F	14.4 (1.59-130.82)	0.018

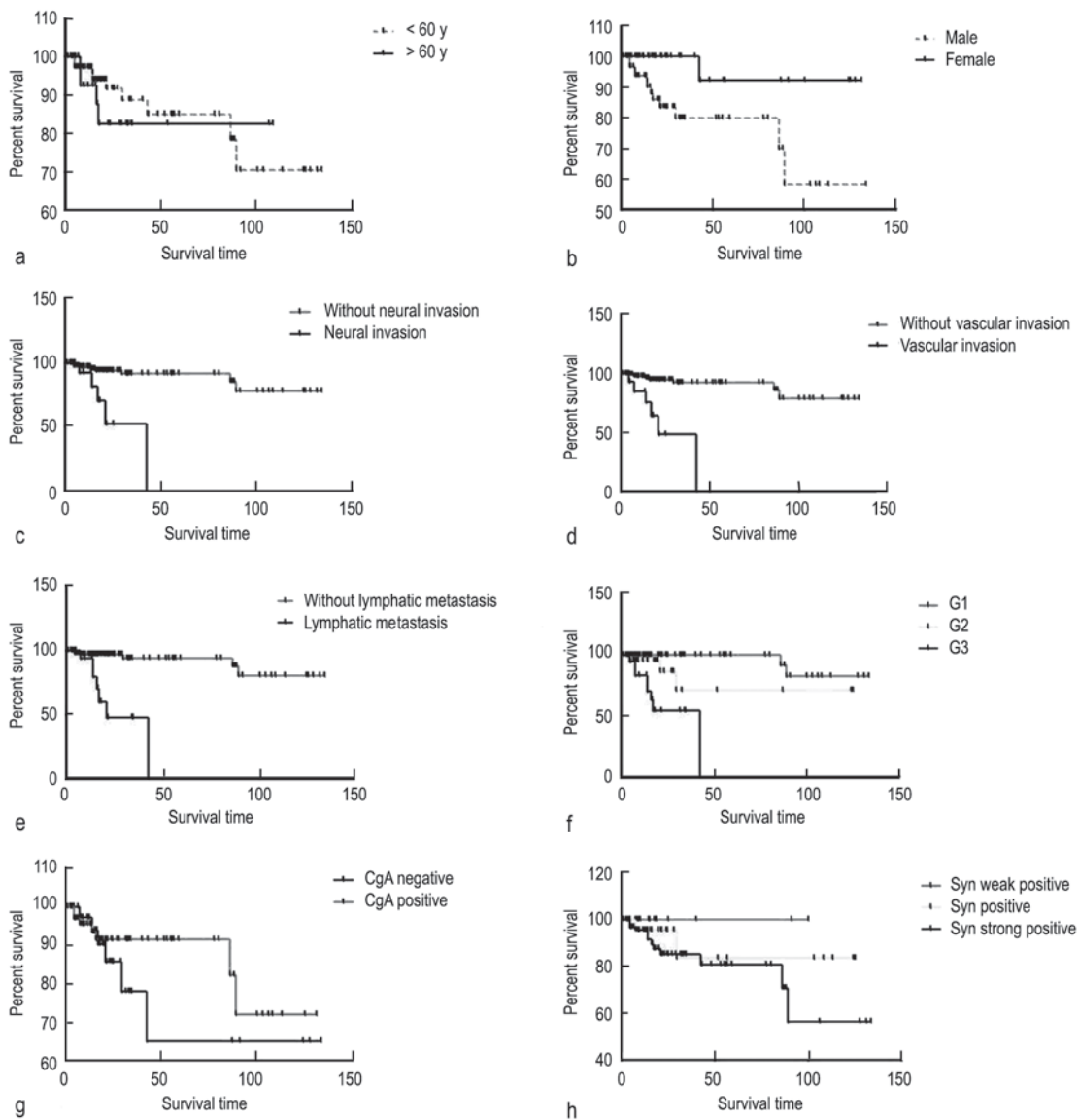


Fig. 4 Survivorship curves of GEP-NEN patients for different pathological parameters. (a) Overall survival among different ages showed no significant difference ($P=0.575$); (b) Overall survival between different gender was statistically significant ($P=0.016$); (c) Overall survival between different neural invasion groups had statistically significant differences ($P<0.001$); (d) Overall survival between different vascular invasion groups with statistical difference ($P=0.005$); (e) Overall survival difference between different lymph node metastasis groups were statistically significant ($P<0.001$); (f) Overall survival between different grading had significant statistical significance ($P<0.001$); (g) The survival time among different CgA expression intensities had no significant difference ($P=0.85$); (h) The survival time had no significant difference among different Syn expression intensities ($P=0.95$)

invasion and patients with high-level GEP-NEN were relatively high-risk, as shown in Table 2.

The pathological grade of GEP-NEN was performed according to mitotic figure and/or Ki-67 positive index [3] (Table 3).

The majority of well differentiated NET were G1 or G2 tumors, while G3 tumor was most poor differentiation of NEC. After analyzing between the tumor pathological grade and clinical pathological parameters, statistically significant difference ($P<0.001$) in nerve vascular invasion, lymph node metastasis and tumor size in different

tumor grading, different groups of age and sex had no significant difference (Table 4).

Discussion

In recent years, epidemiology shows that the incidence of GEP-NEN indicated an increased trend [2, 5-6]. Our country has not built the whole national cancer registration system, lack of GEP-NEN authority, epidemiological data and the overall incidence [7]. Incidence of NEN is not

Table 3 Gastrointestinal and pancreatic neuroendocrine tumor grading standards

Classification	Proliferation activity level by mitosis (10 HPF)	Ki-67 positive index (%)
G1	1	≤ 2
G2	2–20	3–20
G3	> 20	> 20

Table 4 The relationship between tumor pathological grade and clinical pathological parameters

Pathological features	G1 (76)	G2 (24)	G3 (19)	χ^2	<i>P</i>
Nerve infiltration					
Y	1	4	9		
N	75	20	10	31.75	< 0.001
Lymph node metastasis					
Y	0	2	15		
N	76	22	4	78.24	< 0.001
Vascular infiltration					
Y	1	4	9		
N	75	20	10	31.75	< 0.001
Age (years)					
< 60	56	16	8		
≥ 60	20	8	11	6.88	0.032
Sex					
M	46	10	16		
F	30	14	3	8.03	0.018
Size of tumor (cm)					
≤ 2	61	9	1		
> 2	15	15	18	41.66	< 0.001

low according to the current domestic NEN information [8]. This ratio of male to female in this group was 1.53:1, which is similar to Europe and the United States reports [2]. Median age was 53 years, morbidity peak age ranging from 50 to 59 years old, followed by 40–49 years old and 60–69 years old, the data is consistent with the United States [2].

Hassan *et al* [9] found that women are more likely to suffer from NEN than men at a earlier onset age regardless of pathogenic sites due to the susceptibility genes. In this research, the difference between male and female patients is similar to Hassan's study. Women's average onset age is younger than that of men, thus indicating women may have the earlier onset age. However, the specific mechanism remains to be further determined. GEP-NEN can occur in any part of the digestive system. Guo *et al* [10] summarized from 863 domestic GEP-NEN literature and pointed out that in China GEP-NEN tends to occur in pathogenic sites as follows: the pancreas (49.8%), rectum (24.3%) and the appendix (11.1%). In this research, the author found out that pancreas NEN took up the highest proportion (37.82%), followed by rectum (27.73%) and stomach (21.85%). While in Western countries, the result is quite different, GEP-NEN often occur in the intestinal

parts [2, 11]. The study showed that the difference in pathogenic sites may be related to sample numbers and racial differences [2, 12].

The symptoms are closely related to pathogenic sites. The gastrointestinal NEN has no obvious different clinical symptoms from those of gastrointestinal tract cancers [13], such as, epigastric ache, epigastric discomfort, and black stool, difficulty in swallowing, angular. The primary symptom of patients with rectal NEN is defecate habit change [14–16]. In this study, gastrointestinal tract NEN also indicates the same clinical pathological symptoms. In this experiment, the major proportion is non functional GEP-NEN, only a few of insulin tumor and somatostatin tumor appears similar symptoms, in agreement with reported data [17]. GEP-NEN patients showed no specific clinical manifestations, thus endoscopic, B ultrasonic, CT are used as important diagnostic methods. Undoubtedly, histopathological and immunohistochemical methods are one of the main methods, with its advantage of indicating the relationship and degree of infiltration between tumor tissue and adjacent organs, blood vessels and nerve.

Immunohistochemical results indicate that Syn expression between G2 and G1, G3 tumor has significant difference, and Syn expression in G2 tumor is significantly higher than in G1 and G3, clinical pathological diagnosis of Syn not only can be used as an important neuroendocrine markers, but also can do the tumor classification according to Syn and Ki-67 expressions. Because of the small sample size, the sample should be expanded to further research of this study.

Survival analysis of this research showed that the GEP-NEN patients' age, gender, presence of vascular invasion, nerve invasion, with and without lymph node metastasis and tumor grade have significant differences on the patients' overall survival time. On the basis of single factor analysis, multi-factor Cox regression analysis was performed and showed that nerve invasion is an independent risk factor for the prognosis of patients. Once the other independent risk factors were under control, the risk of death of patients with nerve invasion was 4.23 times the rest of the patients. Gender also affect the prognosis of patients, the risk of death in male patients is 14.40 times higher than women. Gender analysis showed that the proportion of males in G3 grade (16/19) was significantly higher than women (3/19). This may also due to the poor prognosis in men. This showed that patients with neurological involvement and male patients may be high risk group who need extra attention. This result was not reported before, therefore, further study is needed. Tumor G3 has a poor prognosis and is an independent risk factor, which is similar to former reports [18].

Some reports, such as Yao [2] conducted multi-factor analysis to raise the point that TNM staging, pathological type, Ki-67, immunohistochemical markers CgA and Syn

were factors which could affect the prognosis of patients. However our research showed no significant difference of CgA and Syn expressions between the neuroendocrine tumor and neuroendocrine carcinoma, so the two markers could not be used for neuroendocrine tumor prognosis, this view remains to be confirmed. Further analysis for G1, G2, and G3 showed that patients with nerve and vascular invasion, lymph node metastasis, gender, age and tumor size at different levels of the NEN had remarkable difference in the incidence. And as the grade raised, nerve and vascular invasion, lymph node metastasis, and the incidence of large tumors (> 2 cm) are significantly increased, which also reveals a poor prognosis in high-level NEN.

Surgical treatment is one of the main radical treatment for patients with early NEN^[19-20]. This group of patients received surgical treatment. For late recurrence and transfer of the NEN, there are no effective treatments. Although in our study, a small number of patients accepted the chemotherapy and radiotherapy, there is no standard treatment. In addition to chemotherapy, biological treatment such as octreotide biological treatment could inhibit disease pathogenesis by controlling carcinoid syndrome^[21]. The small molecular targeted therapy drugs such as Choungny brought hope for the NEN treatment, which can be applied to patients of G1 and G2^[22].

In conclusion, the GEP-NEN can occur in any part of the digestive system, lack of specific clinical manifestations, mainly depends on the pathological diagnosis and surgery is the main way of treatment. This study found that the NEN often occurs in pancreas, rectum and stomach tumor grade, nerve vascular invasion, gender are important factors. For judging prognosis, also, detection of CgA, Syn has clinical significance in the differential diagnosis of neuroendocrine tumor cells.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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