Int J Clin Exp Med 2017;10(3):4897-4904
www.ijcem.com /ISSN:1940-5901/IJCEM0045112

Original Article

Clinical features of patients with pancreatic neuroendocrine neoplasms: a retrospective analysis of 61 cases in a cancer center

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Received August 2, 2016; Accepted February 5, 2017; Epub March 15, 2017; Published March 30, 2017

Abstract: Objective: To assess the clinicopathological features of patients with pancreatic neuroendocrine neoplasms (pNENs) in relation with clinical outcomes. Methods: A total of 61 patients with pNENs were enrolled in this study. Patients' characteristics, treatment procedures, and clinical follow-up were retrospectively analyzed. Results: Among the 61 patients, 29 patients (47.5%) were men and 32 patients (52.5%) were women. 6 patients (9.8%) had functional pNENs and presented with diarrhea, flushing, sweating, palpitations or glucose abnormalities. At diagnosis, 31.1% (19/61) had a localized disease, 6.6% (4/61) presented with regional spread including lymph node metastases and/or extrapancreatic organ invasions, and 62.3% (38/61) had distant metastases. Liver was the most common site of metastasis. 58 cases were recorded with detailed pathological information, among which 42 patients (72.4%) were diagnosed as NET G1/G2, 16 patients (27.6%) were diagnosed as NEC G3. The chromogranin A (CgA) levels were elevated in the serum of patients with liver metastasis, unresectable tumors or a Ki-67 proliferative index ≥20%. The univariate analysis suggested that the WHO classification, stage, occurrence of liver metastasis and operative approaches were closely related to the prognosis of pNENs; while sex, age, tumor size and functional status were not. Conclusion: The pNENs are a heterogeneous group of neoplasms with diverse clinical manifestations and prognosis. Serum CgA is an indicator of tumor burden and proliferation. The WHO classification, stage, liver metastasis and operative approaches might closely relate to the prognosis of pNENs.

Keywords: Pancreatic, neuroendocrine neoplasm, diagnosis, treatment, serum CgA

Introduction

Pancreatic neuroendocrine neoplasms (pNENs) are a group of heterogeneous neoplasms that may be derived not only from mature pancreatic endocrine cells but also from pluripotent stem cells of the pancreas. According to the Surveillance, Epidemiology and End Results (SEER) study [1], pNENs are considered rare, with an annual incidence of 1-2 of every 100,000 individuals; these tumors account for 1-2% of all pancreatic tumors. The natural history of this entity is difficult to predict at diagnosis. Furthermore, little data is available on the epidemiology and survival of patients with pNENs in China. Prognostic factors have varied markedly in different studies of pNENs and which factors are important in Chinese patients with pNENs have not yet been identified.

Chromogranin A (CgA) is a 49-kDa acidic glycoprotein, which is a principal component of dense-core granules in neuroendocrine cells. CgA is secreted from neuroendocrine cells during the secretory granule exocytosis and also used as a circulating marker [2]. Previous studies have shown that elevated serum CgA levels were demonstrated in patients with pNENs. It has also been suggested that serum CgA may be a biomarker for predicting recurrences and monitoring the follow-up [3].

Due to the heterogeneity of pNENs, findings from previous studies, including long-term out-

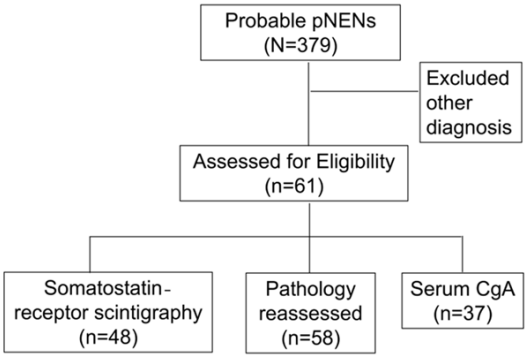

Figure 1. The flowchart of the study and distribution of patients recruited.

comes, prognostic factors and serum CgA cut-off value, might not be relevant for Chinese patients with pNENs. Therefore, we reviewed the clinicopathological features of pNENs at a single center and analyzed the treatment, survival and prognosis in these patients.

Material and methods

Patient selection

In the present study, the clinical data of all 61 consecutive patients who were histologically diagnosed with pNEN from May 2009 to June 2015 at Beijing Cancer Hospital was collected. Patients without pathologic confirmations of pNEN or with primary tumors in other sites were excluded from this study. A functional tumor was defined as a tumor-overproducing hormone that caused clinical symptoms. This study was approved by the medical ethics committee of Peking University Cancer Hospital and written consent was provided for patient information to be used for research purposes.

Histopathology and serum CgA

The consensus diagnosis was based on pathological morphology and immunohistochemical staining of surgical specimens or tumor biopsies performed by pathologists. The World Health Organization (WHO) 2010 classification, which is a widely accepted grading system, was adopted in this study. According to the classification system, tumors were classified into low grade (G1), intermediate grade (G2), or high grade (G3) [4]. The categories were based upon mitotic count and proliferative index (Ki-67 staining). The Ki-67 index was calculated as a percentage of Ki-67 positive cells in 2000 neoplastic cells in areas of strongest nuclear labeling. Mitotic count was based on counting 50 high-power fields and in the area of highest mitotic activity and reported as the number of mitoses per 10 high-power fields. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues. Immunohistochemistrical stainings, including chromogranin A (CgA) and synaptophysin (Syn), were performed to detect the biochemical markers of pNENs.

Peripheral blood samples were collected from patients with pNENs and centrifuged (2500 g) at 4°C for 10 minutes after blood clotting to obtain serum. Serum CgA level was detected using an enzyme-linked immunosorbent assay (ELISA) kit (ChromoaTM, Cisbio) according to the manufacturer's instructions. All serum samples were measured in triplicate. (**Figure 1**).

Follow-up and survival

Follow-up was performed from June to October 2015 by telephone or outpatient visit. The duration of overall survival (OS) was measured from the date of diagnosis until tumor-specific death or the latest follow-up. The relapse time was calculated from the date of remission to recurrence. We excluded few patients who died of other causes when selecting the experimental subjects.

Statistical analysis

Statistical analyses were performed using IBM SPSS 19.0 statistical software. Data was presented as the mean ± the standard error of the mean unless otherwise indicated. Categorical variables were presented as numbers and their frequencies are presented as proportions. Pearson's χ^2-test and Fisher's exact test were used to compare proportions when appropriate. Kaplan-Meier estimates of the survival rates were plotted, and differences in survival distribution between stages were evaluated by the log-rank test. The analysis of risk factors was performed by univariate analyses by the Cox proportion hazards method. Two-sided *P* values <0.05 were considered statistically significant.

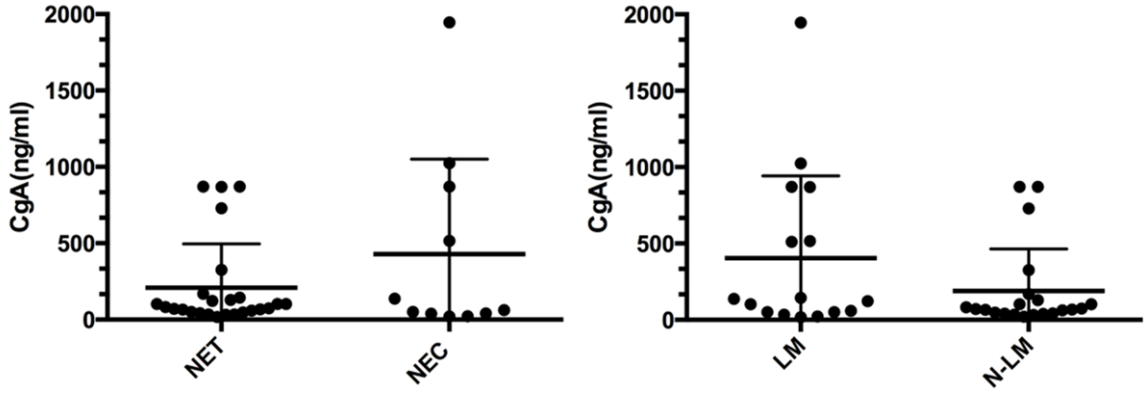

Figure 2. Serum CgA levels in relation to the tumor grade of pNENs and liver metastasis. CgA, chromogranin A; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; LM, liver metastases; N-LM, non-liver metastases.

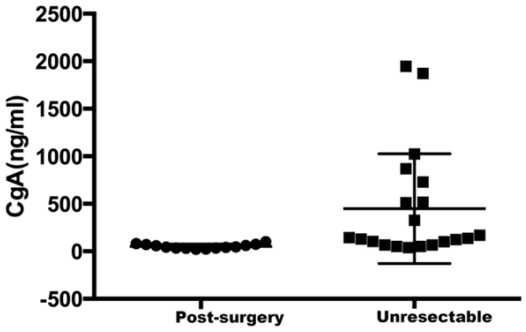

Figure 3. Serum CgA in patients with post-surgery and unresectable disease.

Results

Patient characteristics

Among the 61 patients with pNENs, 29 patients (47.5%) were men and 32 patients (52.5%) were women. The age at diagnosis ranged from 21 to 76 years old (mean ± SD: 51.92±12.46). In total, 55 patients (90.2%) had nonfunctional pNENs; 6 patients (9.8%) had functional pNENs presented with diarrhea, flushing, sweating, heart palpitations or glucose abnormalities.

Out of 58 cases with thorough pathological data, 8 patients (13.8%) were diagnosed with NET G1, 34 patients (58.6%) were NET G2, and 16 patients (27.6%) were NEC G3. In the NEC G3 group (n=16), 17.5% of patients had tumors which exhibited Ki-67 index greater than 50%. The positive rates of CgA and Syn were 86.2% and 89.7%, respectively.

A total of 31.1% (19/61) of patients had localized disease, 6.6% (4/61) developed regionally advanced tumors, and 62.3% (38/61) had distant metastases. Liver was the most common site of metastasis, the rates of metastasis of G1, G2, G3 tumors were 55.5%, 53.1% and 93.8%, respectively.

Serum CgA was detected in 37 patients. The elevated level of circulating CgA was demonstrated in the serum of patients with liver metastasis (405.0±134.2 vs. 189.4±59.84, $P<0.05$) (**Figure 2**), whereas the level of serum CgA in patients after surgery was lower than that in patients with unresectable tumors (51.9±6.41 vs. 489.4±129.4, $P<0.0001$) (**Figure 3**). Somatostatin-receptor scintigraphy was performed in 48 patients, and 40 patients (83.8%) has positive result. The positive rates of patients with NET and NEC were 81.8% and 86.7%, respectively (**Table 1**).

Treatment and prognosis

Nineteen patients with localized disease underwent radical surgery while 14 patients with locally advanced or metastatic disease received palliative surgery. A total of 73.37% (14/19) patients experienced recurrence or distant metastatic spread after radical surgery; the median recurrence free survival was 34.1 months.

Twenty-eight patients with unresectable metastatic disease received medical therapy including systemic chemotherapy (platinum-based combination regimen, capecitabine plus temozolomide, and S-1 plus oxaliplatin), somatostatin analogs and molecularly targeted therapy (sunitinib, sulfatinib and everolimus).

Table 1. Clinical and pathological Features of the Entire Cohort with pNENs

Classification	Number of patients (%)
Sex	
Male	29 (47.5%)
Female	31 (52.5%)
Age at diagnosis, y	
Mean ± SEM	51.92±12.46
Range	21~84
Somatostatin-receptor scintigraphy	
All	83.3% (40/48)
NET	81.8% (27/33)
NEC	86.7% (13/15)
Tumor Grade	
NET G1	8 (13.8%)
NET G2	34 (58.6%)
NEC G3	16 (27.6%)
Immunohistochemistry	
CgA	86.2% (50/58)
Syn	89.7% (52/58)
Stage	
Localized	19 (31.1%)
Regional	4 (6.6%)
Metastatic	38 (62.3%)
Metastatic sites	
Liver	31 (52.5%)
Lymph nodes	7 (11.5%)
Lung	3 (4.9%)
Bone	4 (6.5%)
Others	3 (4.9%)

NET: neuroendocrine tumor; NEC: neuroendocrine carcinoma.

The 1-, 2-, and 5-year overall survival (OS) rates were 92.4%, 77.3% and 61.1%, respectively. The univariate analysis suggested that the WHO classification, stage, occurrence of liver metastasis and operative approaches were closely related to the prognosis; while sex, age, tumor size and functional status were not (**Table 2**; **Figure 4**).

Discussion

In this study, 90.2% patients had nonfunctional pNENs (NF-pNENs) with nonspecific manifestations, which is relatively higher than the proportion of NF-pNENs (75%~85%) reported by an epidemiology study from the United States [5]. This could be partly explained by the nature of our center, which is a tertiary cancer center. Patients with hormone-related symptoms are more frequently diagnosed and treated at general hospitals in China. SEER data indicated that the most favorable outcomes were observed in patients with benign tumors, in females, in younger patients and in those with functional tumors [6]. However, in our study we did not find that sex, age, tumor size and functional status were correlated with prognosis. This inconsistency may due to the limited sample size in our study.

The optimal cut-off value for baseline serum CgA remains unknown [7], and an early study has reported a 51.2% sensitivity and an 87.5% specificity by setting 95 ng/ml as the cut-off value [8]. Our findings indicate that serum CgA is an indicator of tumor burden, as evidenced by the increased CgA level found in patients with liver metastasis, unresectable tumors or a tumor with Ki-67 proliferative index ≥20%. Given the significant role of serum CgA in the prediction of the prognosis of patients, we suggest that a serum CgA level ≥95 ng/ml is associated with a worse prognosis.

Somatostatin-receptor scintigraphy (SRS) was applied using 99Tc-HYNIC-Tyr3-OCT or 68Ga-labeled somatostatin analogues as the imaging agent for single photon emission tomography (SPETCT) or positron emission tomography (PET), which potentially detect more tumor sites during staging and the follow-ups. In our cohort, the positive rates of NET and NEC were comparable (>80%).

According to the 2010 WHO classification, the majority of patients in our study were diagnosed with pNETs. The prognosis of NECs was far worse than that of NETs, and high-grade tumors were often associated with a worse prognosis. Unlike the NECs that showed a marked expression of Syn and focal or weakly positive expression of CgA, NETs often exhibited diffuse and strong CgA and Syn expression. The significance of immunohistochemistry test in the prediction of the survival in patients with pNENs is still debatable, as studies have found that the simultaneous expression of Syn and CgA suggested a better prognosis, whereas positive CD117 expression served as a potential predictor of worse survival [9]. Whether patients whose tumors express SSTR2a have a

Table 2. Univariate Analysis of Predictors for pNENs

Variable	Number	OS, Median, mo	χ^2	*P* valve
Sex				
Female	24	NR	1.294	0.255
Male	21	55.670		
Age				
<50 y	23	NR	0.069	0.792
≥50 y	22	67.470		
Function				
Yes	5	NR	0.125	0.724
No	40	67.470		
Tumor size				
≤4 cm	17	NR	0.128	0.721
>4 cm	22	67.470		
Serum Cga(ng/ml)				
Cga<95	19	NR	6.175	0.013
Cga≥95	18	61.230		
Tumor grade				
G1	5	61.230	6.685	0.035
G2	25	NR		
G3	15	27.670		
Classification				
NET	31	NR	4.889	0.027
NEC	14	27.670		
Tumor Stage				
Local	15	NR	9.713	0.008
Regional	3	67.470		
Metastatic	27	39.670		
Distal metastasis				
No	18	NR	9.327	0.002
Yes	27	39.670		
Liver Metastasis				
Yes	23	39.670	9.703	0.002
No	22	NR		
Surgery				
Yes	25	NR	11.998	0.001
No	20	39.670		

NR: not reached.

prolonged survival requires further investigations [10].

Aggressive surgery is recommended for patients with localized disease or resectable tumors who received neoadjuvant therapy [11]. In our study, 73.68% patients experienced recurrence or distant metastases after radical surgery. The recently published data from ASCO in 2015 showed a recurrence rate of 26% in pNENs (n=141) and a median follow-up of 62.1 months after R0 resection [12]. In our study, the median recurrence free survival was 34.1 months, and the difference between our study with the ASCO report is probably caused by the bias of patient population treated in our hospital. The patients enrolled in the current study tend to be in an advanced stage or have heavy tumor burden. Recent literatures showed that the peak of recurrence was 2 years after surgery [13]. Therefore, close monitoring and follow-up for a longer period of time seem imperative.

Distant metastasis is associated with a significantly worse prognosis. In our study, a total of 62.3% patients had distant metastases at the time of diagnosis, and liver was the most common site of metastases. Radical surgery can be performed in only 10% patients with diffuse and multiple liver metastases [14]. Even for unresectable tumors, palliative debulking of liver metastases can effectively alleviate symptoms caused by compression or excessive hormone secretion, and can prolong overall survival and tumor-specific survival [15]. Although liver metastasis is associated with a worse prognosis, in our study we found that resection of the primary tumor and palliative surgery of metastases could prolong survival. Even when liver metastasis occurred after radical surgery, the resection of liver lesions was still beneficial for survival.

In this study, patients with unresectable tumors received chemotherapy, biological therapy or molecularly targeted therapy and exhibited a worse survival compared with those who underwent radical surgery or palliative surgery. There is still no standard regimen [16] for patients with unresectable tumors. The objective response rate of streptozotocin combined with 5-FU and epirubicin was 35%~40%, and the majority of patients could not tolerate the toxicity associated with this regimen [17]. Platinum-based regimens were mainly applied to pNECs [18]. There is still a lack of evidence for temozolomide alone or in combination with capecitabine, irinotecan, oxaliplatin or other targeted drug regimens [19, 20]. Currently, sunitinib and everolimus have been recommended for

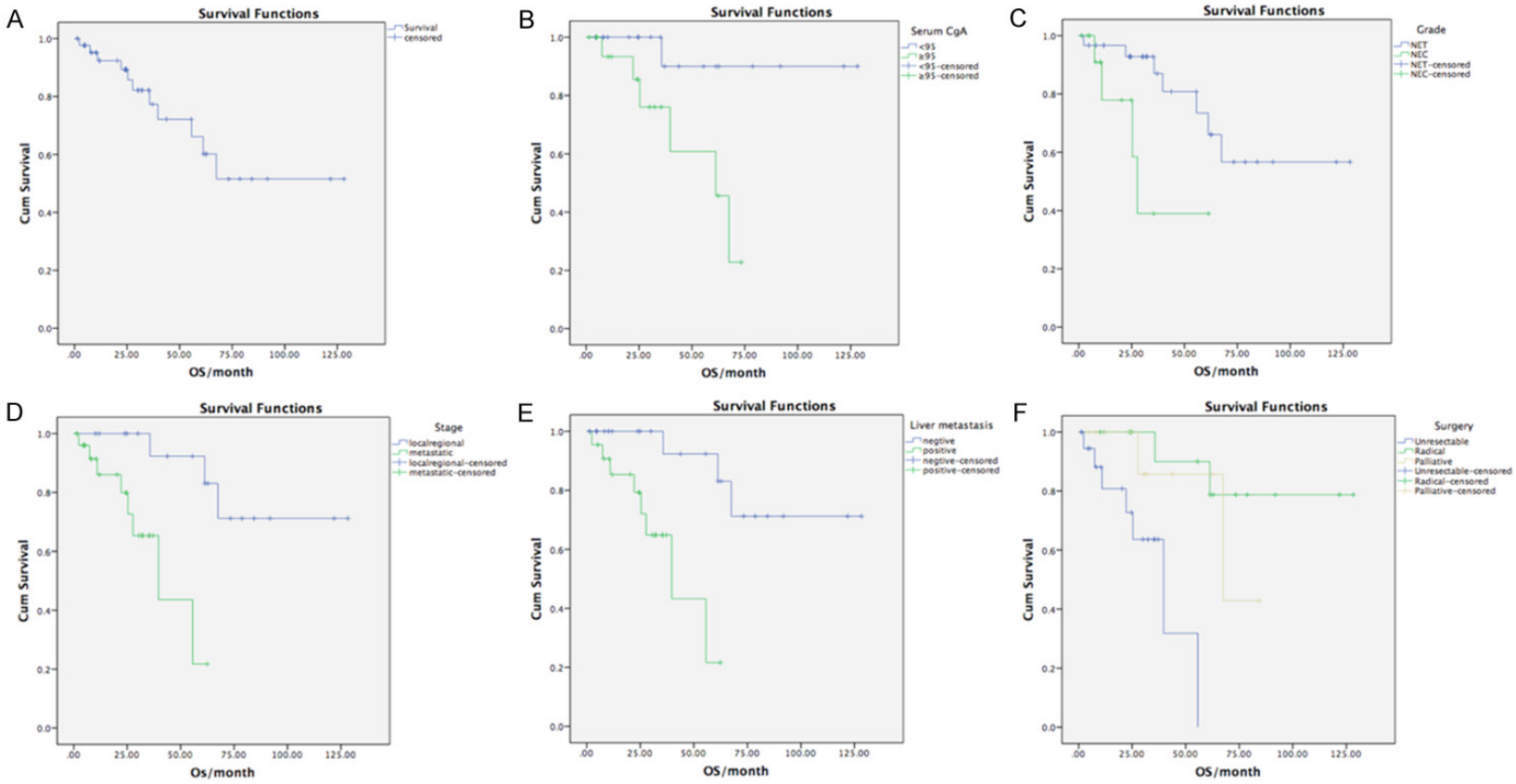

Figure 4. The overall survival of patients with pNENs (A). Comparison of survival among patients of serum CgA (B), grade (C), stage (D), liver metastasis (E) and surgery approaches (F). OS, overall survival.

advanced well-differentiated pNETs, but the side effects may affect the patients' quality of life [21, 22].

In conclusion, pNENs are a heterogeneous group of neoplasms with diverse clinical manifestations and prognoses. Serum CgA could be used as an indicator of tumor burden and proliferation. The WHO classification, stage, liver metastasis and operative approaches might closely relate to the prognosis of pNENs. A multidisciplinary approach that incorporates chemotherapy, surgery, biological targeted therapy and interventional therapy should be employed in primary care, but the symptoms, stage, histology, volume of metastatic disease, and growth rate also deserve consideration.

Disclosure of conflict of interest

None.

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