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Original article

Microscopic venous invasion in patients with pancreatic neuroendocrine tumor as a potential predictor of postoperative recurrence☆

Yoshihide Nanno [a], Hirochika Toyama [a, *], Kyoko Otani [b], Sadaki Asari [a], Tadahiro Goto [a], Sachio Terai [a], Tetsuo Ajiki [a], Yoh Zen [b], Takumi Fukumoto [a], Yonson Ku [a]

[a] Department of Surgery, Kobe University Graduate School of Medicine, Kobe, Japan
[b] Department of Diagnostic Pathology, Kobe University Graduate School of Medicine, Kobe, Japan

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ABSTRACT

Background: Microscopic venous and lymphatic invasion is a known prognostic factor for various cancers, but its prognostic relevance for pancreatic neuroendocrine tumors (PNETs) is unclear.
Methods: Thirty-two consecutive patients with PNET who had complete resection were included in this study. Venous and lymphatic invasion was identified on elastic tissue or immunohistochemical staining, and correlated with other clinicopathological factors, including recurrence-free survival.
Results: Venous and lymphatic invasion was identified in nine (28%) and three (9%) patients, respectively. Tumors with venous invasion were of significantly larger size, higher Ki-67 index, and higher mitotic counts. Patients with venous invasion showed significantly worse prognosis than those without venous invasion ($P = 0.001$). Five of nine patients (56%) with venous invasion had tumor recurrence, while a relapse was found in one case in patients without venous invasion ($n = 23$). Lymphatic invasion was not correlated with any other clinicopathological parameters including lymph node metastasis and recurrence-free survival. Predictive factors for recurrence in univariate analysis included microscopic venous invasion, tumor size $\geq$ 20 mm, non-functionality, and WHO grades. In multivariate analysis where WHO grades and microscopic venous invasion were applied, venous invasion remained a significant predictor of poor recurrence-free survival ($P = 0.021$).
Conclusions: Microscopic venous invasion may serve as a predictive factor for tumor recurrence in patients with resectable PNET. The combination of WHO grades and microscopic venous invasion may assist in the stratification of the patients for risk of tumor recurrence.

1. Introduction

Pancreatic neuroendocrine tumor (PNET) is a rare form of epithelial neoplasm with histologic evidence of neuroendocrine differentiation. This unique neoplasm has been increasingly diagnosed in Japan and other countries [1,2]. PNETs are classified into two groups based on histopathological findings. Well differentiated PNETs are characterized by relatively uniform, less dysplastic tumor cells arranged in a solid or trabecular pattern, while poorly differentiated PNETs are pancreatic equivalents of lung small cell carcinomas or large cell neuroendocrine carcinomas [3]. As mitotic counts and Ki-67 indices on tissue are currently the only reproducible prognostic factors for patients with PNET, these microscopic findings are currently endorsed for tumor grading by the World Health Organization (WHO) [4]. In the 2010 WHO classification, PNETs are graded on a three-tiered system [2,5]. Although grade 3 (G3) PNETs are highly malignant neoplasms with aggressive clinical behavior, it is generally difficult to predict the prognosis of patients with G1/2 PNET. Identification of additional prognostic factors that can be used together with WHO grading will assist in the stratification of patients for the risk of tumor recurrence.

Lymphatic and venous drainage are primary routes of tumor dissemination from a primary tumor site to regional lymph nodes

☆ **Institute where the work was conducted**: Kobe University Hospital.

* Corresponding author. Department of Surgery, Kobe University Graduate School of Medicine, 7-5-2, Kusunoki-cho, Chuo-ku, Kobe, 650-0017, Japan.
E-mail address: toyama@med.kobe-uc.ac.jp (H. Toyama).

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and distant organs. The presence of microscopic venous invasion is believed to be a histologic indicator of aggressive tumor behavior, and has been determined as an independent factor for poor prognosis for various cancers [6–8]. Previous studies on neuroendocrine tumors (NETs) of the lung has shown that microscopic venous and lymphatic invasion is significantly correlated with WHO histologic grades, tumor sizes, recurrence, and patients' survival [9]. However, this correlation remains controversial for PNETs. In the current study, we examined microscopic venous and lymphatic invasion in patients with resectable well differentiated PNET using immunohistochemistry for CD31 and D2-40, and correlated the results with other clinicopathologic parameters including recurrence-free survival.

2. Methods

2.1. Patient selection

The study protocol was approved by the Ethics Committee at Kobe University, and written informed consents were obtained from all patients. During the study period from January 2008 to April 2014, 33 patients with PNET underwent surgical resection at our institute. One patient was excluded from this study due to the presence of multiple small metastatic nodules in the liver found during surgery that lead to incomplete resection. The remaining 32 patients who had complete resection were included in this study. In our facility, standard pancreatectomy was primarily the treatment of choice. Organ/parenchymal-sparing resection could be considered for small tumors (<20 mm) without any signs of metastasis. Patients were carefully explored for metastasis with manipulation and intraoperative ultrasonography, followed by regional lymph node dissection or sampling with or without intraoperative frozen sections. Following the standard, surgical procedures performed were: subtotal stomach-preserving pancreatoduodenectomy ($n = 8$), pylorus-preserving pancreatoduodenectomy ($n = 1$), distal pancreatectomy ($n = 9$), spleen-preserving distal pancreatectomy ($n = 2$), middle pancreatectomy ($n = 2$), and enucleation ($n = 10$). An additional liver resection was simultaneously performed in one patient with synchronous liver metastasis.

2.2. Examination of clinical features

Medical records and imaging of the selected patients were retrospectively reviewed. The location and extent of disease were determined based on pre-operative imaging, including ultrasonography, enhanced computed tomography (CT), magnetic resonance imaging (MRI), endoscopic ultrasonography (EUS), and endoscopic retrograde cholangiopancreatography. Positron emission computerized tomography (PET) was also performed in 24 patients (75%). Tumor sizes were calculated based on the last enhanced CT prior to surgery.

2.3. Pathological examination

Histology slides of the 32 cases were retrospectively reviewed by two experienced pathologists (KO and YZ). Microscopic venous invasion was assessed using CD31 immunostaining and Elastica van Gieson staining [10]. Lymphatic vessels were visualized by immunostaining for D2-40 [6]. As D2-40 is highly specific for the lymphatic endothelium, neoplastic cells floating in vessels lined by D2-40-positive cells were considered as lymphatic invasion, while tumor-cell nests surrounded by an elastic lamina or CD31-positive and D2-40-negative endothelium were counted as venous invasion. Ki-67 labeling indices were re-evaluated using GunmaLI [11], an image analytical software which enables automatic quantification of Ki-67 indices. One representative section was selected for these stainings. For one patient who had multiple PNETs, the largest tumor was chosen for the histologic examination.

2.4. Follow-up data

Three patients with G1 insulinoma (12, 12, 9 mm, respectively) were followed at our outpatient clinic with repeated CT scans every 12 months. The remaining patients were followed with repeated CT scans every 3–6 months. Additional MRI, EUS, PET studies and/or histological biopsy were performed upon suspected recurrence. The median follow-up period after surgery was 46 months (range, 5–86 months). During follow-up, two patients died of PNETs, and three patients died of other causes. The 1-, 3-, and 5-year overall survival rates after curative resection were 97%, 89%, and 78%, respectively.

2.5. Statistics

Categorical variables were compared using Fisher's exact test for comparison between two groups, and Pearson's chi-squared test for three or more groups. A two-sided P value of less than 0.05 was considered statistically significant. For the follow-up analysis, recurrence-free survival was evaluated in this study, as PNETs are slow-growing neoplasms requiring longer follow-up periods for analyzing patients' survival. Recurrence-free survival was determined from the date of operation to diagnosis of recurrence. Patients without recurrence were censored at the last visit. Survival curves were estimated using the Kaplan-Meier method and compared using the Log-rank test. Univariate and multivariate survival analyses were also performed using Cox proportional hazard models. A Cox proportional hazard analysis was used to estimate hazard ratios and 95% confidence intervals with adjustment for the effects of the other pathologic factors. P values of less than 0.05 were considered statistically significant. All analyses were performed with JMP 11.0 for Macintosh (SAS Institute, Inc., Cary, NC).

3. Results

3.1. Clinical features

The study cohort consisted of 18 men and 14 women with a median age of 64 years (range: 29–87 years). The tumor sizes ranged from 10 to 156 mm (median 16 mm). Three patients had another primary NET in an extrapancreatic organ (the lung, duodenum, and stomach). Two patients had a history of von Hippel-Lindau disease, and another patient had multiple endocrine neoplasia type 1.

Based on WHO grading, 16 cases were graded as G1, and 13 were G2. The remaining three showed well differentiated morphological features, but Ki-67 indices appeared to be >20% (20.6%, 30.6%, and 33.9%), therefore the PNETs were diagnosed as G3 grade. Although this study aimed to examine G1/2 well differentiated PNETs, the three G3 PNET cases were not excluded as they were considered as borderline neoplasms (PNETs with well differentiated morphology but a Ki-67 index > 20%).

3.2. Microscopic venous and lymphatic invasion in PNETs

Nine patients (28%) had microscopic venous invasion, while three (9%) showed lymphatic invasion (Fig. 1). Venous invasion was identified inside tumor masses or fibrous capsules on immunostaining and Elastica van Gieson staining.

Table 1 summarizes the correlations between microscopic