

Survivals of patients with pancreatic neuroendocrine carcinomas

An in-depth analysis by the American Joint Committee on Cancer 8th tumor-node-metastasis staging manual

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Abstract

Recently, the American Joint Committee on Cancer (AJCC) 8th staging manual stipulated the World Health Organization (WHO) G3 pancreatic neuroendocrine carcinomas (p-NECs) should all be classified by the system for pancreatic exocrine adenocarcinomas, which had ignored the heterogeneity of G3 p-NECs. We focused on demonstrating whether the heterogeneous subgroups of G3 p-NECs would influence the accurate application of AJCC 8th staging systems.

G3 p-NECs were divided into well-differentiated and poorly-differentiated subgroups, whose clinical features and overall survival (OS) were compared. Survival analysis by applying 2 new AJCC 8th staging systems to well-differentiated G3 p-NECs were performed to validate whether these subgroup patients should also be staged by the system proposed for all G3 p-NECs.

We enrolled 172 patients who were histopathologically diagnosed as G3 p-NECs, including 64 well-differentiated G3 p-NECs and 108 poorly-differentiated ones, whose patient demographics and tumor characteristics present no notable differences ($P > .05$), except their Ki-67 index and mitotic rate ($P = .031$, $P = .025$; respectively). The estimated OS of well-differentiated G3 p-NECs was significantly better than those of poorly-differentiated tumors ($P < .001$). When applying the new AJCC system for all G3 p-NECs to well-differentiated G3 tumors, 18, 22, 12, and 12 patients were respectively distributed in the new AJCC Stage I, Stage II, Stage III, and Stage IV. Using the AJCC 8th staging system for WHO G1/G2 pancreatic neuroendocrine tumors (p-NETs) to well-differentiated G3 p-NECs, there were 5, 25, 22, and 12 patients classified from the new AJCC Stage I to Stage IV, respectively. The system for G1/G2 p-NETs could significantly differentiate the survival differences between each new stage of well-differentiated G3 p-NECs ($P < .05$), while comparisons of survivals between Stage II with Stage III or Stage III with Stage IV by the system for G3 p-NECs were not statistically different ($P = .334$, $P = .073$; respectively).

G3 p-NECs were heterogeneous with well-differentiated and poorly-differentiated subgroups. Both AJCC 8th staging systems proposed for all G3 p-NECs and G1/G2 p-NETs were practical for well-differentiated G3 p-NECs, while the one originally applied to G1/G2 p-NETs appeared to be superior in performance due to its better prognostic stratification and more accurate predicting ability.

Abbreviations: AIC = Akaike information criterion, AJCC = American Joint Committee on Cancer, C-index = Harrell's concordance index, CIs = confidence intervals, ENETS = European Neuroendocrine Tumor Society, HPFs = high power fields, HRs = Hazard ratios, K-M = Kaplan-Meier, MST = median survival time, OS = over survival, p-EACs = pancreatic exocrine adenocarcinomas, p-NECs = pancreatic neuroendocrine carcinoma, p-NENs = pancreatic neuroendocrine neoplasms, p-NETs = pancreatic neuroendocrine tumors, TNM = tumor-node-metastasis, WHO = World Health Organization.

Keywords: AJCC, pancreatic neuroendocrine carcinomas, prognosis, staging, well-differentiated

1. Introduction

Pancreatic neuroendocrine neoplasms (p-NENs), namely islet cell tumors, are a group of highly heterogeneous tumors with different clinical manifestations, pathological features,

biological behaviors and long-term prognosis.^[1,2] Accounting for 2% to 3% of all pancreatic malignancies, p-NENs are uncommon, with an increasing trend of incidence in the past three decades, probably due to the development of imaging

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technology and the improvement of people's awareness for p-NENs.^[3,4]

The ability to classify p-NENs into prognostic groups have always been challenging due to the rarity and heterogeneity of this disease. Histologically, the World Health Organization (WHO) formally classified p-NENs into 3 main subgroups in 2010 based on the mitotic rate and Ki-67 index of tumor cell: G1 or G2 pancreatic neuroendocrine tumors (p-NETs) and G3 pancreatic neuroendocrine carcinoma (p-NECs).^[5] This grading classification for p-NENs derived from the one by the European Neuroendocrine Tumor Society (ENETS) in 2006 and were widely used thereafter.^[6] Clinically, in 2010, the American Joint Committee on Cancer (AJCC) also distributed p-NENs into 4 stages based on the tumor-node-metastasis (TNM) characteristics (ie, the 7th edition): localized tumors (stage I), locally advanced but resectable tumors (stage II), locally advanced and unresectable tumors (stage III), and distantly metastasized tumors (stage IV), which was originally applied to pancreatic exocrine adenocarcinomas (p-EACs).^[7]

In 2017, the AJCC updated its 8th staging manual for all solid tumor, in which a new and specific TNM staging system for p-NENs was firstly introduced.^[8] Meanwhile, some important changes in the 8th manual have been incorporated. Firstly, the WHO 2010 grading classification for p-NENs was thoroughly applied to the new systems, which emphasized the significance of tumor grading when staging p-NENs. Secondly, the 8th staging system for p-NENs should only be applied to the G1/G2 p-NETs, which was analogue to the system proposed by ENETS in 2006. Thirdly, the G3 p-NECs should be grouped by the 8th staging system for p-EACs, which also incorporated some major changes differing from its 7th one.

The new AJCC 8th staging systems for p-NENs have been a tremendous and significant progress for further cancer research, especially for different grading subgroups, while their clinical value has seldom been evaluated. Our previous studies have respectively validated the applications of 2 new systems for G3 p-NECs and G1/G2 p-NETs, which both concluded each system could better classify eligible patients into prognostic groups than the AJCC 7th edition staging system.^[9,10] Interestingly, we identified 2 subgroups of G3 p-NECs with notably different survivals, which might reveal their heterogeneous behaviors.^[9] Meanwhile, accumulative studies have already reported that G3 p-NECs consist of morphologically well-differentiated and poor-differentiated tumors with different clinical features and long-term prognosis.^[11-15] On the other hand, however, the AJCC 8th staging system for G3 p-NECs regarded all G3 p-NECs as an entirety and staged all these tumors by using the new system for p-EACs.^[8] This regulation ignored the heterogeneity of G3 p-NECs, which might be the potential defect of AJCC 8th staging manual for p-NENs. In the present research, based on our previous studies referring to the AJCC 8th TNM systems for p-NENs,^[9,10] we made an in-depth analysis for the survivals of G3 p-NECs. We first described the clinical characteristics of 2 morphologically different G3 p-NECs subgroups. We then emphasized to validate how the heterogeneity of G3 p-NECs would influence the accurate application of AJCC 8th staging systems when stratifying G3 p-NECs. As far as we know, our study was the first attempt to do such a thing, which might provide some potential theory for the improvement of next AJCC 9th TNM staging manual for p-NENs, especially for G3 p-NECs.

2. Materials and methods

2.1. Patient enrollment

Patients who were histopathologically diagnosed as G3 p-NECs at West China Hospital of Sichuan University from January 2002 to December 2018 were enrolled in the present study. Differing from our previous study in which patients underwent an operation or a resection,^[9,10] patients with biopsy by ultrasound-guided fine needle aspiration were also enrolled in order to acquire as many cases as possible. For included cases, relevant data was retrospectively collected as we ever did.^[9,10] Our study was also approved by the Institutional Review Board and Ethics Committee of the West China Hospital of Sichuan University. Written informed consent was obtained on admission from all patients, in accordance with the general principles of the Helsinki Declaration.^[16]

2.2. Tumor features

According to the WHO 2010 grading classifications, G3 p-NECs were defined as having >20 mitotic figures per 10 high power fields (HPFs) or a Ki-67 index of >20%.^[5] Then, we defined G3 p-NECs into two morphologically different subgroups in the light of some recognized histopathological features^[5,14,17]: Well-differentiated G3 p-NECs were marked by typical neuroendocrine architectural tissues with organoid features and tumor cells with low nucleocytoplasmic ratio, abundant eosinophilic or amphophilic cytoplasm, and ovoid nuclei with salt and pepper chromatin containing well-defined nucleoli; Poorly-differentiated G3 p-NECs were featured on nodular or solid architecture lack of organoid traits, usually with high nucleocytoplasm ratio and multifocal or extensive tumor necrosis, including small cell and large cell subtypes. The TNM staging systems by AJCC 8th staging manual were performed for all included patients mainly based on the results of preoperative image examinations, intraoperative surgical findings, and postoperative pathological diagnosis.^[8] In the present study, we emphasized to evaluate the influence of the heterogeneity of G3 p-NECs on straiting these patients into prognostic groups by AJCC 8th staging manual. To accomplish this goal, we performed the analysis by applying two new AJCC systems (one for G1/G2 p-NETs and the other for G3 p-NECs) to well-differentiated G3 p-NECs subgroup to validate whether these patients should also be staged by the system proposed for all G3 p-NECs (or for p-EACs).

2.3. Follow-up and survival

Follow-up was mainly performed by office visit, telephone call, e-mail or outpatient clinic visit from May 2019 to July 2019. There was a total of 32 patients who were lost to follow-up and were censored in the final survival analysis, including 9 patients with well-differentiated G3 p-NECs and 23 ones with poorly-differentiated tumors. Over survival (OS) was calculated as the time elapsed from the date of diagnosis to the date of death from any cause or last follow-up visit.

2.4. Statistical analysis

The statistical analysis in the present study was the same as we ever did in our previous research^[9,10]: Quantitative variables were reported as medians with ranges, while categorical

variables were presented as numbers with frequencies and proportions (%). OS was estimated using Kaplan-Meier (K-M) methods and compared using the log-rank test. Univariate and multivariate analyses by the Cox regression proportional hazards model were performed separately to validate the predictive value of the 2 new AJCC 8th staging systems for the OS of well-differentiated G3 p-NECs. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated for each variable on multivariate analysis. Weighted Cohen kappa coefficients were computed to evaluate the inter-rater agreement of 2 new systems for well-differentiated G3 p-NECs. When comparing the prognostic accuracy of both classifications, the Akaike information criterion (AIC) and the Harrell concordance index (C-index) were respectively calculated in the Cox regression model. A smaller AIC or a larger C-index value indicated a better model for predicting outcome. Statistical analyses were performed using Statistical Package for Social Science version 22.0 software (SPSS Inc., Chicago, IL). A *P* value less than .05 was considered statistically significant.

3. Results

3.1. Patient demographics and tumor characteristics

According to the inclusive criteria we defined above, we finally enrolled 172 eligible patients who were histopathologically diagnosed as G3 p-NECs, including 64 well-differentiated G3 p-NECs and 108 poorly-differentiated ones. Table 2 showed the patient demographics and tumor characteristics of all G3 p-NECs in the present study. As it listed, there were no notable statistical differences when comparing factors between well-differentiated G3 p-NECs and poorly-differentiated tumors, such as patients' gender and age, tumor location and type, incidental diagnosis, diagnostic date and approach, preoperative imaging examinations, operative data, postoperative medical therapy, etc (*P* > .05). Similarly, although the tumor diameter of well-differentiated G3 p-NECs was smaller than poorly-differentiated ones (4.5 cm vs 5.6 cm) and the proportion of patients with well-differentiated G3 p-NECs who underwent a R0 resection seemed to be larger than those with poorly-differentiated tumors (82.5% vs 70.7%), their differences were also not statistically significant (*P* = .059, *P* = .078; respectively). The Ki-67 index and mitotic rate of well-differentiated G3 p-NECs were both notably smaller than those of poorly-differentiated tumors (36 vs 64, 22 vs 41; *P* = .031, *P* = .025; respectively).

3.2. Stages of well-differentiated G3 p-NECs by 2 AJCC 8th staging systems

Table 1 showed the present analysis of two AJCC 8th TNM staging systems for well-differentiated G3 p-NECs. When applying the new AJCC system for G3 p-NECs to well-differentiated G3 tumors, 6, 15, 26, and 17 patients were respectively defined from T1 to T4 stage. 16 patients were pathologically confirmed to have 1 to 3 regional lymph nodes metastases (N1 stage), while 8 ones had 4 or more regional lymph nodes metastases (N2 stage). Twelve patients present distant metastases at diagnosis (M1 stage). As for the related clinical stages, 18, 22, 12, and 12 patients were respectively distributed in the new AJCC Stage I, Stage II, Stage III, and Stage IV. When applying the AJCC 8th staging system for G1/G2 p-NETs to well-differentiated G3 p-NECs, there were respectively 5, 11, 27, and

21 patients who were defined from T1 to T4 stage. There was a total of 24 patients with regional lymph node metastasis (N1 stage) and 12 ones with distant metastases at diagnosis (M1 stage). In terms of the corresponding clinical stages, there were 5, 25, 22, and 12 patients classified from the new AJCC Stage I to Stage IV, respectively.

3.3. Survivals of well-differentiated and poorly-differentiated G3 p-NECs

When the follow-up ended, there were 84 deaths, including 28 patients with well-differentiated G3 p-NECs and 56 ones with poorly-differentiated G3 p-NECs. As a result, the estimated OS at 3 and 5 years for the whole group of G3 p-NECs was 42.7% and 21%, respectively, with a median survival time (MST) of 31.9 mon (Fig. 1). With respect to the different subgroups of G3 p-NECs, the estimated 3- and 5-year OS of well-differentiated G3 p-NECs was respectively 63.1% and 38.8% with a MST of 51.3mon, which was significantly better than those of poorly-differentiated G3 p-NECs (25.1%, 4.7%, 26.4 mon; *P* < .001; Fig. 2).

3.4. Survivals of well-differentiated G3 p-NECs by 2 AJCC 8th staging systems

When applying the AJCC 8th staging system for G3 p-NECs to well-differentiated G3 p-NECs, there were 6, 7, 6, and 9 deaths from Stage I to Stage IV. The estimated 3-year OS for each new stage was 94.1%, 74.9%, 44.4%, and 10%, respectively, with a MST of 74.2 mon, 47.8 mon, 32.3 mon, and 21.5 mon (*P* < .001; Fig. 3). Comparisons of survivals between Stage I with Stage II or Stage III or Stage IV were all statistically significant (*P* = .039, *P* < .001, *P* < .001; respectively), as well as those between Stage II with Stage IV (*P* = .001); while those between Stage II with Stage III or Stage III with Stage IV were not (*P* = .334, *P* = .073; respectively). In terms of the new AJCC system for G1/G2 p-NETs, 1, 9, 9, and 9 patients with well-differentiated G3 p-NECs in each new stage were respectively dead when the follow-up ended. The estimated OS at 3 years from Stage I to Stage IV was 100%, 90.6%, 49.2%, and 10%, respectively, with a MST of not applicable (NA), 57.3 mon, 32.3 mon, and 21.5 mon (*P* < .001; Fig. 4). Comparisons of survivals between Stage I with Stage II or Stage III or Stage IV were also statistically significant (*P* = .021, *P* = .029, *P* = .003; respectively), as well as those between Stage II with Stage III or Stage IV (*P* = .015, *P* < .001; respectively) and that between Stage III with Stage IV (*P* = .023).

3.5. Prognostic analyses for well-differentiated G3 p-NECs

As Table 3 demonstrated, patients' gender, tumor location, and incidental diagnosis were not predictive for the OS of well-differentiated G3 p-NECs (*P* > .05). Meanwhile, patients' age, tumor diameter and type and postoperative medical therapy were only statistically significant in univariate analyses (*P* < .05), while radical resection, AJCC 8th staging systems proposed for either G3 p-NECs or G1/G2 p-NETs were all prognostic factors for the OS of well-differentiated G3 p-NECs in both univariate and multivariate analyses (*P* < .05). In the Cox regression proportional hazards model, the 95% CIs of AJCC 8th staging system for G1/G2 p-NETs (0.823–1.574) were smaller than that of AJCC system for G3 p-NECs (0.615–2.153), indicating a relatively more accurate predictive ability.

Table 1
The original definitions of 2 AJCC 8th TNM staging systems for p-NENs and present analysis of 2 comparable criteria for well-differentiated G3 p-NECs* (N=64).

AJCC 8th staging system for G3 p-NECs		AJCC 8th staging system for G1/G2 p-NETs			
T/N/M staging definitions — (Cases)					
T1	Tumor 2 cm or less in greatest dimension — (6);	Tumors limited to pancreas, 2 cm or less in greatest dimension — (5);			
T2	Tumor more than 2 cm but no more than 4 cm in greatest dimension — (15);	Tumors limited to pancreas more than 2 cm but less than 4 cm in greatest dimension — (11);			
T3	Tumor more than 4 cm in greatest dimension — (26);	Tumors limited to pancreas, more than 4 cm in greatest dimension or tumors invading duodenum or bile duct — (27);			
T4	Tumor involves coeliac axis, superior mesenteric artery and/or common hepatic artery — (17).	Tumors perforates visceral peritoneum (serosa) or invades other organs or adjacent structures — (21).			
N0	No regional lymph node metastasis — (40);	No regional lymph node metastasis — (40);			
N1	Metastases in 1 to 3 regional lymph nodes — (16);	Regional lymph node metastasis — (24).			
N2	Metastases in 4 or more regional lymph nodes — (8).	NA.			
M0	No distant metastasis — (52);	No distant metastasis — (52);			
M1	Distant metastasis — (12).	Distant metastasis — (12).			
Clinical staging definitions — (Cases)					
Stage I	T1 N0 M0 (A) — (6); T2 N0 M0 (B) — (12);	T1 N0 M0 — (5);			
Stage II	T3 N0 M0 (A) — (14); Any T N1 M0 (B) — (8);	T2 N0 M0 (A) — (9); T3 N0 M0 (B) — (16);			
Stage III	Any T N2 M0 — (4); T4 Any N M0 — (8);	T4 N0 M0 (A) — (10); Any T N1 M0 (B) — (12);			
Stage IV	Any T Any N M1 — (12).	Any T Any N M1 — (12).			
Cross-tabulation of two AJCC 8th staging systems — (Cases)					
For G1/G2 p-NETs	Stage I	Stage II	Stage III	Stage IV	Total
For G3 p-NECs					
Stage I	5	13	0	0	18
Stage II	0	12	10	0	22
Stage III	0	0	12	0	12
Stage IV	0	0	0	12	12
Total	5	25	22	12	64

AJCC=American Joint Committee on Cancer, G=grading, M=distant metastasis, N=regional lymph node, NA=not applicable, p-NECs=pancreatic neuroendocrine carcinomas, p-NENs=pancreatic neuroendocrine neoplasms, p-NETs=pancreatic neuroendocrine tumors, T=primary tumor, TNM=tumor-node-metastasis.

*In the present study, we emphasized to evaluate the influence of the heterogeneity of G3 p-NECs on stratifying these patients into prognostic groups. So, we applied 2 new AJCC 8th TNM staging systems (one for G1/G2 p-NETs and the other for G3 p-NECs) to well-differentiated G3 p-NECs to validate whether these patients should also be staged by the system proposed for all G3 p-NECs.

3.6. Assessments between two AJCC 8th staging systems for well-differentiated G3 p-NECs

As the cross-tabulation performed in Table 1, patients grouped in Stage I (n=18) and Stage II (n=22) by the AJCC 8th staging criteria for G3 p-NECs were respectively distributed to Stage I (n=5) or Stage II (n=13) and Stage II (n=12) or Stage III (n=10) in the light of the new system for G1/G2 p-NETs. Patients classified in Stage II (n=25) and Stage III (n=22) according to the AJCC 8th staging criteria for G1/G2 p-NETs were respectively distributed to Stage I (n=13) or Stage II (n=12) and Stage II (n=10) or Stage III (n=12) by the new system for G3 p-NECs. The weighted Cohen κ coefficient between 2 new AJCC systems was calculated as 0.612 (95% CIs: 0.421–0.804), indicating a rough agreement and moderate discrepancy ($P=.023$). The AIC value of the AJCC 8th staging system for G1/G2 p-NETs was smaller than that of the system for G3 p-NECs (3056.41 vs 3208.47), while the C-index of the AJCC 8th staging system for G1/G2 p-NETs was significantly larger than that of the system for G3 p-NECs [0.615 (95% CIs: 0.573–0.779) vs 0.501 (95% CIs: 0.468–0.641); $P=.036$]. The consistent results of the C-index and the AIC value indicated that the AJCC 8th staging system for G1/G2 p-NETs appeared to be superior to its system for

G3 p-NECs in terms of the prognostic stratification and informative ability for the OS of well-differentiated G3 p-NECs subgroup.

4. Discussion

P-NENs are a group of uncommon neoplasms with a wide spectrum of biological behaviors from benign to malignant.^[1–4] However, due to the heterogeneous behaviors and epidemiological features of p-NENs, the ability to stratify patients into prognostic groups has been hindered by the absence of a widely accepted classification. In 2010, WHO classified p-NENs mainly into G1 p-NETs, G2 p-NETs, and G3 p-NECs, based on their mitotic rate and Ki-67 index, which has been widely used in clinic ever since.^[5] Subsequently, accumulative studies have reported that compared with G1/G2 p-NETs, G3 p-NECs were more likely inclined to be in late Stage III or IV with a worse prognosis, because of their larger tumor size, local invasion and more regional lymph node or distant metastasis at diagnosis or surgery.^[18–20] We have previously reported that the estimated 5-year OS of G1 p-NETs, G2 p-NETs, and G3 p-NECs could be significantly different, with a range of (82.6–87.8) %, (52.7–70.1) %, and (20.7–25.7) %, respectively.^[9,10,21,22] Therefore,

Table 2
The baseline demographics and tumor features of all G3 p-NECs in the present study.

Factors	Well-differentiated*	Poorly-differentiated*	All	P [†] value
No. of cases, n (%)	64 (37.2%)	108 (62.8%)	172 (100%)	
Gender, female	38 (59.4%)	62 (57.5%)	100 (58.1%)	.167
Age at diagnosis, yrs				.421
Median	52	57	53	
Range	7–71	14–80	7–80	
Tumor diameter, cm				.059
Median	4.5	5.6	5.1	
Range	1.8–7.6	1.6–13.1	1.6–13.1	
Tumor location				.114
Head/uncinate	21 (32.8%)	40 (37.1%)	61 (35.5%)	
Body/tail	43 (67.2%)	68 (62.9%)	111 (64.5%)	
Tumor type				.337
Functional	20 (31.3%)	36 (33.3%)	56 (32.6%)	
Non-functional	44 (68.7%)	72 (66.7%)	116 (67.4%)	
Incidental diagnosis				.579
Yes	14 (21.9%)	25 (23.1%)	39 (22.7%)	
No (symptomatic)	50 (78.1%)	83 (76.9%)	133 (77.3%)	
Diagnostic date				.812
Before 2010	10 (15.6%)	19 (17.6%)	29 (16.9%)	
After 2010	54 (84.4%)	89 (82.4%)	143 (83.1%)	
Diagnostic approach				.469
Resection	40 (62.5%)	65 (60.2%)	105 (61.1%)	
Biopsy [‡]	24 (37.5%)	43 (39.8%)	67 (38.9%)	
Preoperative imaging examinations				
US positive	33/50 (66.0%)	60/78 (76.9%)	93/128 (72.7%)	.093
CT positive	35/45 (77.7%)	45/54 (83.3%)	80/99 (80.8%)	.135
MRI positive	32/38 (84.2%)	48/56 (85.7%)	80/94 (85.1%)	.467
Operative data				
Surgical margin, [§]				.078
R0	33 (82.5%)	46 (70.7%)	89 (84.8%)	
R1/R2	7 (17.5%)	19 (29.3%)	26 (15.2%)	
Surgical procedure				.532
PD	15 (23.4%)	33 (30.6%)	48 (27.9%)	
DP	35 (54.7%)	55 (50.9%)	90 (52.3%)	
LRP	14 (21.9%)	20 (18.5%)	34 (19.8%)	
In-hospital stay				.094
Median	9	12	11	
Range	5–24	6–41	5–41	
Postoperative complications				.853
Yes	12 (18.7%)	23 (21.3%)	35 (32.4%)	
No	52 (81.3%)	85 (78.7%)	137 (79.6%)	
Ki-67 index, (%)				.031
Median	36	64	55	
Range	20–55	25–88	20–88	
Mitotic rate, (per 10HPS)				.025
Median	22	41	36	
Range	20–32	34–56	20–56	
Postoperative medical therapy	29 (45.3%)	61 (56.5%)	90 (52.3%)	.502
Dead at follow-up	28 (43.75%)	56 (51.8%)	84 (48.8%)	.083
MST, months	51.3	26.4	31.9	<.001

CT = computed tomography, DP = distal pancreatectomy, G = grading, HPS = high power fields, LRP = local resection of pancreas, MRI = magnetic resonance imaging, MST = median survival time, PD = pancreatoduodenectomy, p-NECs = pancreatic neuroendocrine carcinomas, SD = standard deviation, US = ultrasound.

*Well-differentiated and poorly-differentiated G3 p-NECs were respectively defined according to the recognized histopathological features of these tumors, as we detailedly described in the text.

†Comparisons of factors between well-differentiated and poorly-differentiated G3 p-NECs wherever possible.

‡Including biopsy by transabdominal surgery and those by ultrasound-guided fine needle aspiration.

§"R0 resection" means a negative surgical margin in both microscopical and gross pathological examination, in which negative explorations for both local lymph node and distant organ were simultaneously performed., while R1/R2 means a positive surgical margin in either microscopical or gross pathological examination.

||Including traditional chemotherapy and new molecular targeted therapy.

¶32 patients were lost to follow-up, including 9 ones with well-differentiated G3 p-NECs and 23 with poorly-differentiated G3 p-NECs.

suggestions have come into being that different staging systems and treatment strategies should be adopted for various grading subgroups of p-NENs because of their notably different histological behaviors and prognosis.^[18,23,24]

In the light of WHO 2010 grading classification, G1/G2 p-NECs were commonly regarded as well-differentiated tumors, while G3 p-NECs were considered equally to poorly-differentiated ones.^[5] However, recent studies have further focused on the

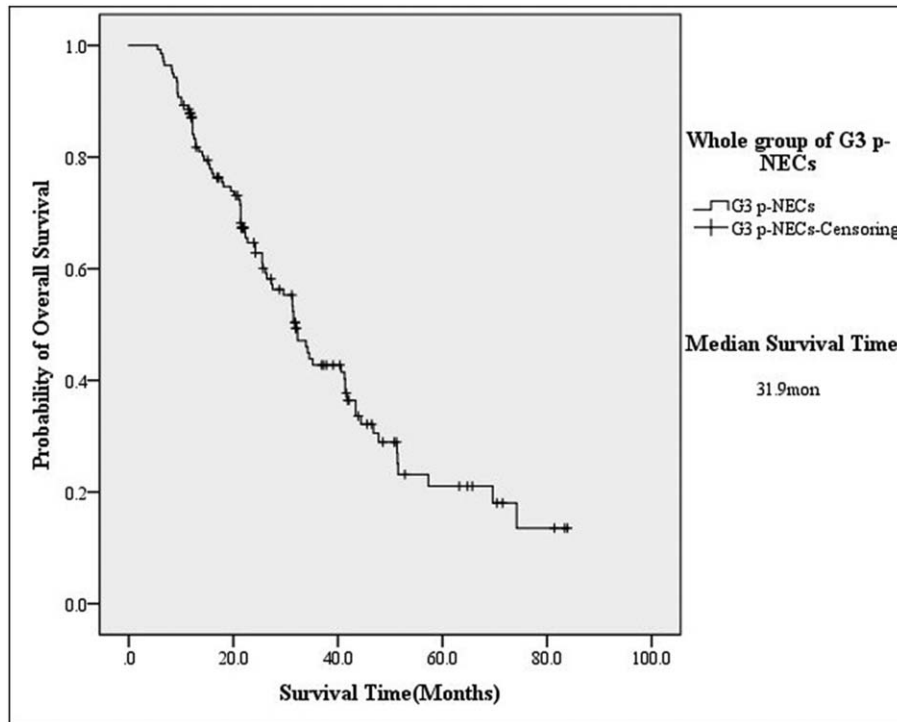


Figure 1. Kaplan-Meier estimates for over survival of the whole group of G3 p-NECs. p-NECs = pancreatic neuroendocrine carcinoma.

significantly heterogenous behaviors within G3 p-NECs, in which some tumors could originally present a high proliferative activity (ie, Ki-67 index) but be morphologically well-differentiated with a better OS. Sorbye et al reported G3 p-NECs were

composed of well-differentiated tumors that usually had a Ki-67 index <55% and did not respond to platinum-based chemotherapy, and poorly-differentiated ones that had a Ki-67 index ≥55% and responded well to platinum-based chemotherapy.^[11,12]

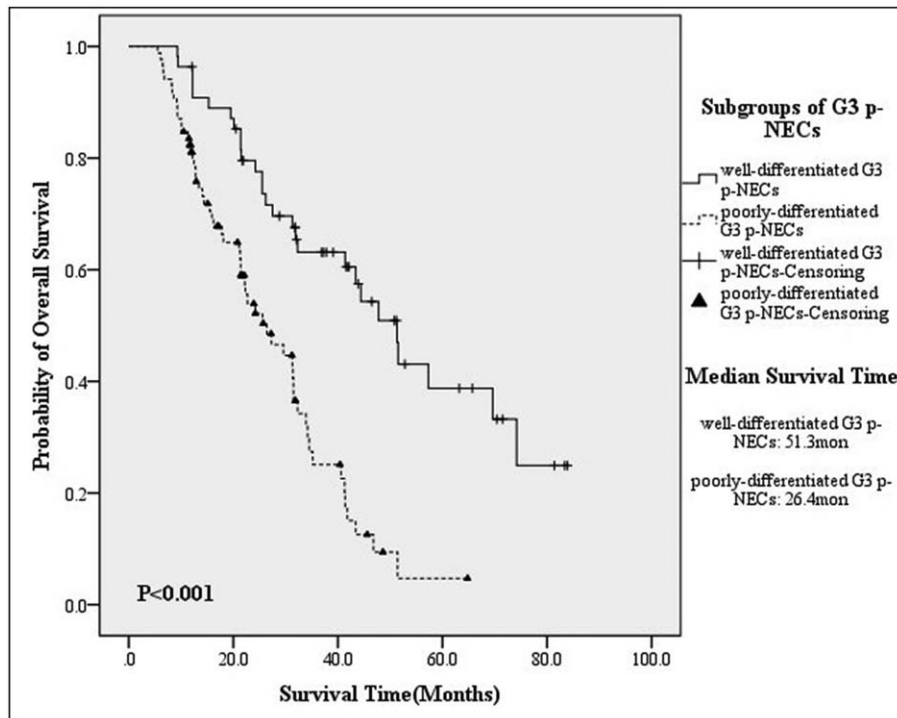


Figure 2. Kaplan-Meier estimates for over survival of the 2 subgroups of G3 p-NECs. p-NECs = pancreatic neuroendocrine carcinoma.

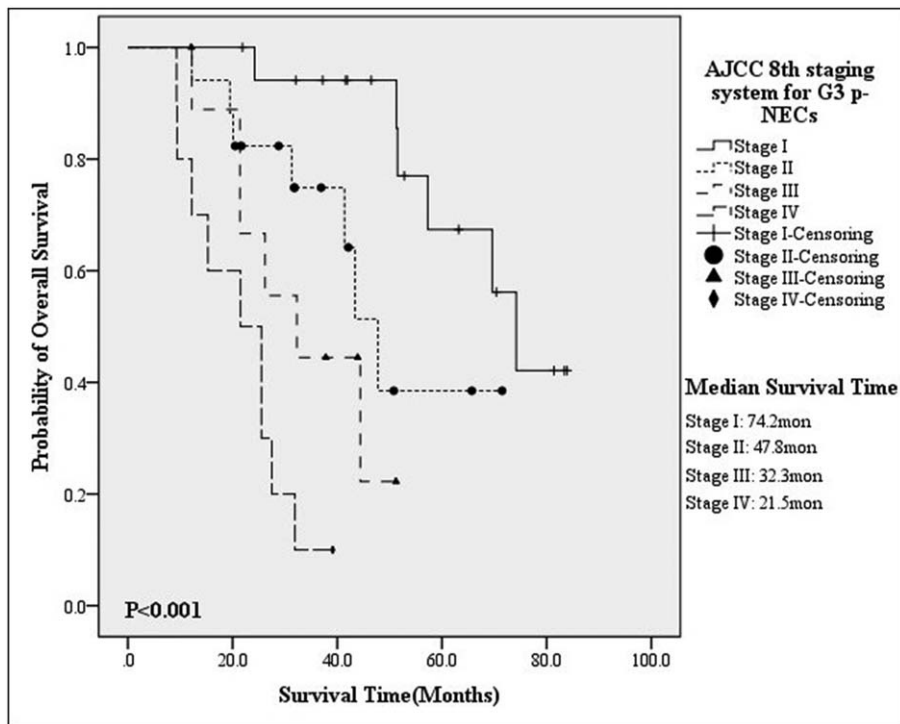


Figure 3. Kaplan-Meier estimates for over survival of well-differentiated G3 p-NECs, according to the AJCC 8th staging system for all G3 p-NECs. AJCC = American Joint Committee on Cancer, p-NECs = pancreatic neuroendocrine carcinoma.

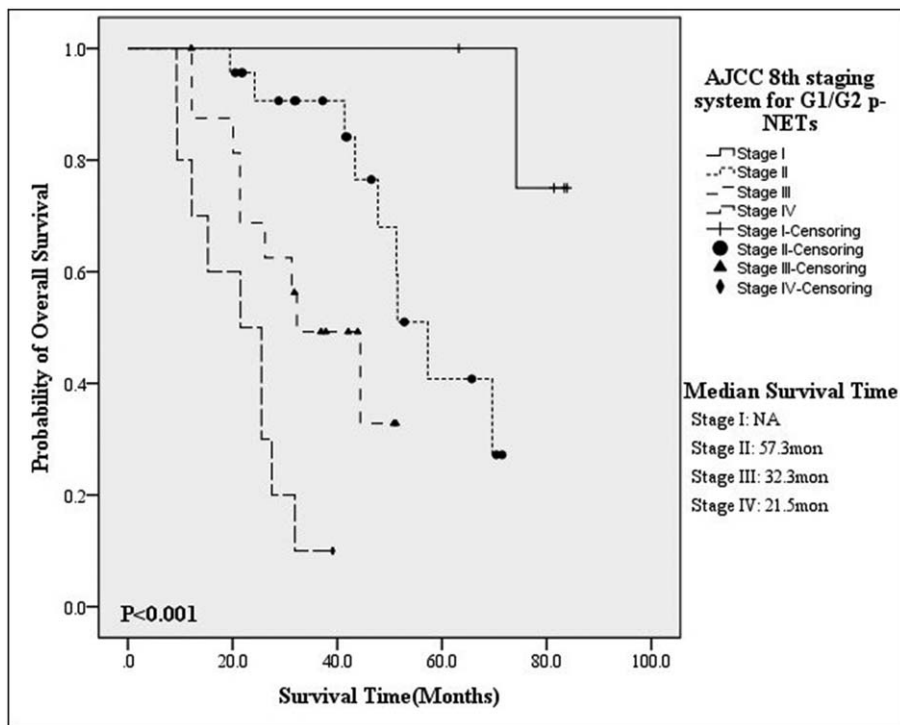


Figure 4. Kaplan-Meier estimates for OS of well-differentiated G3 p-NECs, according to the AJCC 8th staging system for G1/G2 p-NETs. AJCC = American Joint Committee on Cancer, NETs = pancreatic neuroendocrine tumors.

Table 3
Univariate and multivariate analysis of prognostic factors for well-differentiated G3 p-NECs.

Variable	MST (mon.)	Univariate analysis			Multivariate analysis* with AJCC 8th staging system for G3 p-NECs			Multivariate analysis* with AJCC 8th staging system for G1/G2 p-NETs		
		HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Gender										
Male	44.2									
Female	51.1	1.363	0.812–2.035	.125						
Age, yr										
<Median	50.2									
≥Median	32.8	0.340	0.114–0.418	.047	1.323	0.916–1.268	.468	1.118	0.926–1.036	.752
Tumor location										
Head/uncinate	43.4									
Body/tail	51.1	1.253	0.867–2.265	.426						
Tumor diameter										
<Median	52.3									
≥Median	38.2	0.231	0.158–0.397	.025	1.135	0.982–1.674	.512	1.572	0.894–1.542	.832
Tumor type										
Functional	49.7									
Non-functional	38.9	0.683	0.536–0.894	.046	0.683	0.561–1.335	.235	0.773	0.617–1.135	.253
Diagnosis										
Incidental	41.4									
Symptomatic	47.6	2.894	0.863–1.854	.535						
Radical resection										
Yes	49.8									
No	21.2	0.461	0.324–0.784	<.001	0.627	0.357–0.984	.031	0.526	0.315–0.942	.012
Postoperative medical therapy										
Yes	44.3									
No	32.2	0.516	0.586–1.452	.048	0.562	0.613–0.951	.115	0.782	0.451–0.826	.091
AJCC 8th staging system for G3 p-NECs										
I/II	60.1									
III/IV	29.4	0.614	0.415–1.142	.021	1.354	0.615–2.153	.035			
AJCC 8th staging system for G1/G2 p-NETs										
I/II	58.3									
III/IV	33.6	0.712	0.463–1.113	.009				0.518	0.823–1.574	.041

AJCC=American Joint Committee on Cancer, CI=confidence interval, G=grading, HR=hazard ratio, MST=median survival time, NA=not applicable, p-NECs=pancreatic neuroendocrine carcinomas, p-NETs=pancreatic neuroendocrine tumors.

*The potential prognostic value of stage by AJCC 8th staging system for G3 p-NECs or for G1/G2 p-NETs was demonstrated in separate Cox hazard models.

Basturk et al^[13] and Chen et al^[14] also described the WHO G3 p-NECs were morphologically and biologically heterogeneous and included both well-differentiated and poorly-differentiated neoplasms with different Ki-67 indices and prognosis. Milione et al demonstrated that gastroenteropancreatic G3 NECs represent a heterogeneous group of neoplasms which could be better classified in different prognostic categories using both tumor morphology and Ki-67 index.^[15] We previously also identified 2 subgroups of these tumors^[9]: The MST of G3 p-NECs with a Ki-67 index ≥55% was 22.7 mon, compared significantly than 71.3 mon of those with a Ki-67 index <55% ($P < .001$); Meanwhile, the Ki-67 index (≥55% vs <55%) could be an independent prognostic factor for the OS of G3 p-NECs. The heterogeneous phenomena of G3 p-NECs prompted us to perform an in-depth analysis in the present study. We defined G3 p-NECs into well-differentiated tumors and poorly-differentiated ones according to some recognized histopathological features.^[5,14,17] Similarly,^[11–15] we hereby noticed that there were no significant differences between these 2 subgroups of G3 p-NECs when comparing patient demographics and tumor characteristics ($P > .05$) except their Ki-67 index and mitotic rate ($P = .031$, $P = .025$; respectively). The estimated 3- and 5-year OS of well-differentiated G3 p-NECs was significantly better than those of poorly-differentiated tumors (63.1% vs 25.1%, 38.8%

vs 4.7%, respectively; $P < .001$; Fig. 2), which was consistent with the results of our previous study.^[9]

On the other hand, AJCC has been developing TNM staging guidelines for common solid organ tumors since 1977, while the application of its system for p-NENs has experienced a long-time process. In 2006, Bilimoria et al attempted to use the AJCC 6th staging system to stratify p-NENs.^[25] Then, in 2010, AJCC officially introduced a staging system to p-NENs in its 7th manual.^[7] However, both systems above were originally applied to p-EACs, which were proven to be convenient but too oversimplified due to the 2 different disease features between p-NENs and p-EACs.^[26–28] In 2017, AJCC proposed a specific TNM staging classification for p-NENs in its 8th manual.^[8] Simultaneously, AJCC emphasized that the newly defined 8th staging system for p-NENs should only be applied to G1/G2 p-NETs tumors, while G3 p-NECs should be staged according to criteria for p-EACs, which also incorporated many major changes differing from its 7th one. This content was the most important update of AJCC 8th manual for p-NENs, which just reflected the different biological, clinical and prognostic characteristics of G1/G2 p-NETs and G3 p-NECs.

The new AJCC staging systems for p-NENs have been respectively validated in our 2 previous studies,^[9,10] in which we demonstrated that both systems could better stratify eligible

patients into prognostic groups than the 7th edition. Moreover, for G3 p-NECs, we firstly attempted to compare 2 new defined AJCC staging systems for their OS analysis that were respectively proposed for p-EACs and G1/G2 p-NETs.^[9] By applying the p-EAC AJCC 8th staging system to G3 p-NECs, the estimated 3-year OS for each new stage were 86.7%, 76.0%, 44.5%, and 20.7%, respectively ($P < .001$). According to the G1/G2 p-NETs AJCC 8th staging system, the estimated OS at 3 years of G3 p-NECs for each new stage were 100.0%, 83.6%, 47.1%, and 20.7%, respectively ($P < .001$). Our analysis revealed the new system for p-EACs significantly discriminated the survival difference of G3 p-NECs between Stage I and Stage II ($P = .019$), while the other one for G1/G2 p-NETs could not ($P = .108$). Together with the consistent results of Akaike information criteria and C-index, we concluded that both systems proposed for p-EACs and G1/G2 p-NETs respectively in the AJCC 8th staging manual were prognostic for the OS of G3 p-NECs, while the one originally applied to p-EACs was superior.

However, as we mentioned before, the AJCC 8th staging system for G3 p-NECs regarded all G3 p-NECs as an entirety,^[8] which might ignore the heterogeneity of G3 p-NECs with morphologically different-differentiated subgroups. The analysis results above in our previous study reflected the heterogeneity of G3 p-NECs as well when applying AJCC 8th staging manual to these tumors.^[9] The biological behaviors of poorly-differentiated G3 p-NECs were much close to those of p-EACs,^[11,12] so classification for these tumors should undoubtedly be the new AJCC system applied to p-EACs.^[9] Nevertheless, for well-differentiated G3 p-NECs whose prognosis was notably better than that of poorly-differentiated G3 p-NECs, whether these tumors should also be staged by the new AJCC system for p-EACs has still been unclear. Therefore, we conducted this study for the first time to compare which system (one for G1/G2 p-NETs and the other for p-NECs) might be superior when applied to well-differentiated subgroup of G3 p-NECs.

By applying 2 new AJCC staging systems to well-differentiated G3 p-NECs in the present study, we validated that both systems could successfully stratify eligible patients into 4 prognostic groups. Nevertheless, the system for G1/G2 p-NETs could significantly differentiate the survival differences between each new stage of well-differentiated G3 p-NECs ($P < .05$), while comparisons of survivals between Stage II with Stage III or Stage III with Stage IV by the system for G3 p-NECs (or for p-EACs) were not statistically significant ($P = .334$, $P = .073$; respectively). The Cox regression model revealed the 95% CIs of AJCC 8th staging system for G1/G2 p-NETs were smaller than that of the one for G3 p-NECs [(0.823–1.574) vs (0.615–2.153)], indicating a relatively more accurate predictive ability. Furthermore, in combination with the results of the C-index and the AIC value, we demonstrated that the AJCC 8th staging system for G1/G2 p-NETs was superior to the new system for all G3 p-NECs in terms of their prognostic stratification and informative ability for the OS of well-differentiated G3 p-NECs.

In this study, we firstly reported that patients with well-differentiated G3 p-NECs should better be staged by the AJCC 8th staging system for G1/G2 p-NETs, rather than the one for all G3 p-NECs. However, like some other studies with retrospective nature, our research also had some limitations. Most importantly, p-NENs were naturally a group of uncommon disease; as a subgroup of G3 p-NECs, well-differentiated G3 p-NECs were rather rare. There were limited numbers of certain stage grouped by the AJCC 8th staging system. Meanwhile, although we defined

well- and poorly-differentiated G3 p-NECs according to some recognized histopathological features,^[5,14,17] there were no uniform definitions of these tumors up to now. Therefore, studies with large volumes or uniform criteria in the future were still needed to confirm our results.

5. Conclusion

In a word, both AJCC 8th staging systems proposed for all G3 p-NECs and G1/G2 p-NETs were practical for well-differentiated subgroup of G3 p-NECs, while the one originally applied to G1/G2 p-NETs appeared to be superior in performance due to its better prognostic stratification and more accurate predicting ability. Our results supported the more suitable application of AJCC 8th staging system to well-differentiated G3 p-NECs in clinic and might theoretically prompt the improvement of AJCC 9th staging manual for p-NENs.

Author contributions

In this paper, Yi Zhang designed the work and approved the final submission, Bo-Le Tian and Xu-Bao Liu revised the paper; Jie-Yu Wen, Sheng-Zhong Hou and Min Yang prepared the graphs and tables; Jie-Yu Wen, Yang Chen and Min Yang carried out the literature search and statistical analyses of the study; Ben-yuan Deng performed the data collections and wrote the paper. Yi Zhang orcid: 0000-0003-3641-0629.

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