Neuroendocrinology

Research Article

Neuroendocrinology 2021;111:728–738
DOI: 10.1159/000509709

Received: April 10, 2020
Accepted: June 23, 2020
Published online: June 25, 2020

Prognostic Role of Examined and Positive Lymph Nodes after Distal Pancreatectomy for Non-Functioning Neuroendocrine Neoplasms

Giovanni Guarneri[a, b] Louis De Mestier[c] Luca Landoni[d] Stefano Partelli[b] Sébastien Gaujoux[e] Valentina Andreasi[b] Chiara Nessi[d] Safi Dokmak[a] Michele Fontana[d] Bertrand Dousset[e] Philippe Ruszniewski[c] Claudio Bassi[d] Massimo Falconi[b] Alain Sauvanet[a]

[a]Department of Hepato-Bilio-Pancreatic Surgery, ENETS Centre of Excellence, Beaujon Hospital, APHP, Clichy, France; [b]Pancreatic Surgery Unit, ENETS Centre of Excellence, Pancreas Translational and Clinical Research Centre, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy; [c]Department of Gastroenterology and Pancreatology, ENETS Centre of Excellence, Beaujon Hospital, APHP, Clichy, France; [d]General and Pancreatic Surgery, Pancreas Institute, University of Verona, Verona, Italy; [e]Department of Digestive and Endocrine Surgery, Cochin Hospital, APHP, Paris, France

Keywords
Pancreatic neuroendocrine tumours · Lymph node metastases · Surgery · Distal pancreatectomy · Disease-free survival

Abstract
Background: The most appropriate nodal staging system for non-functioning pancreatic neuroendocrine tumours (NF-PanNETs) remains unclear. Despite some evidence is available for pancreaticoduodenectomy, the adequate nodal staging is still unknown for distal pancreatectomy (DP). The aim of the present study was to evaluate the prognostic impact of the number of positive lymph nodes (PLNs) after DP for NF-PanNETs and to define the minimal number of lymph nodes to be harvested for an appropriate nodal staging. ***Methods:*** Data were retrospectively collected from patients who underwent DP with curative intent (R0-R1) for sporadic well-differentiated NF-PanNETs in 4 European high-volume centres. NF-PanNETs with nodal involvement (N+) were subclassified into N1 (1–3 PLNs) and N2 (4 or more PLNs). Univariate and multivariate analyses of disease-free survival (DFS) were performed. ***Results:*** Of 271 patients in the study, 62 (23%) had nodal involvement (N+). A higher probability of N+ was associated with the following factors: grading, resection margin status, perineural and microvascular invasion, and the number of examined lymph nodes. Three-year DFS rate for N0, N1, and N2 patients was 92, 72, and 50%, respectively ($p < 0.001$). At multivariate analysis, independent predictors of DFS were grading, T stage, presence of necrosis, and nodal status. For patients with ≥12 examined/resected lymph nodes, the N status remained a significant predictor of disease recurrence ($p < 0.001$), while it failed to predict recurrence in patients with <12 lymph nodes exam-

Giovanni Guarneri and Louis De Mestier shared the 1st authorship. Alain Sauvanet, Massimo Falconi, and Claudio Bassi shared the senior authorship.

karger@karger.com
www.karger.com/nen

Giovanni Guarneri
Pancreatic Surgery, ENETS Centre of Excellence, IRCCS San Raffaele
Via Olgettina 60
IT–20131 Milan (Italy)
guarneri.giovanni@hsr.it

ined/resected ($p = 0.116$). ***Conclusions:*** A minimal number of 12 nodes should be harvested in case of DP for NF-PanNET for an appropriate nodal staging. The number of positive lymph nodes is an independent predictor of DFS after DP for NF-PanNET, and the N0/N1/N2 nodal classification seems to be more relevant than the current N0/N+ staging.

Introduction

Pancreatic neuroendocrine tumours (PanNETs) are rare lesions that represent approximately 1–2% of all pancreatic neoplasms [1, 2] with an estimated incidence of nearly 1/100,000 people per year [3]. Radical surgery is the treatment of choice for non-metastatic resectable PanNETs [4, 5]. An adequate lymphadenectomy should be associated with surgery for all PanNETs larger than 2 cm [6]. Several pathological factors have been proposed as predictors of recurrence after surgery for PanNETs, and these factors influence the indication and the extent of surgery [7]. The presence of lymph node metastases (N+) is one of the most powerful predictors of disease recurrence among these factors [8], and it is usually associated with higher grade, T-stage, and larger tumour size [6, 7, 9].

The latest Union for International Cancer Control (UICC) TNM staging system (8th edition) [10] distinguishes only N0 and N+ PanNETs, considering nodal involvement only as present or absent. However, for adenocarcinomas and poorly differentiated neuroendocrine carcinomas (PanNECs), nodal involvement is classified based on the number of positive lymph nodes (PLNs) in N0, N1 (1–3), and N2 (≥4).

Recently, it has been suggested that the number of PLNs is an important prognostic factor also in PanNETs, since it more accurately predicts the risk of recurrence than the N+ status alone [11–15]. These data highlight the importance of performing an adequate lymphadenectomy during pancreatic resection for PanNET. Nevertheless, studies on the minimum number of nodes to be resected are still lacking.

Partelli et al. [12] reported that a minimum number of 13 lymph nodes should be harvested to accurately stage patients who undergo pancreaticoduodenectomy for non-functioning PanNETs (NF-PanNETs). Regarding distal pancreatectomy (DP), only one preliminary retrospective study suggested that a minimum number of 7 examined lymph nodes (ELNs) are necessary for appropriate staging, but the pathological examination was not optimal due to the lack of data regarding perineural and microvascular invasion and the presence of necrosis [16]. Moreover, the potential prognostic role of the lymph-node ratio (LNR) has never been explored in this setting.

The aim of the present study was to determine the minimum number of lymph nodes that should be harvested for appropriate nodal staging after DP performed for NF-PanNETs. The secondary aim was to evaluate the prognostic role of the number of PLNs in this population.

Patients and Methods

Data Collection

All patients who underwent DP for PanNETs between January 2005 and June 2017 in 4 high-volume European institutions (Beaujon and Cochin Hospitals in Paris, France, San Raffaele Hospital in Milan, Italy, and Pancreas Institute of Verona, Italy) were screened for inclusion in this retrospective study. All consecutive patients who underwent DP with curative intent (R0/R1) for a sporadic, histologically proven NF-PanNET were included. A minimum post-operative follow-up of 36 months was required for inclusion. All patients with a genetic syndrome were excluded, as well as patients with functioning PanNETs. All G3 tumours were retrospectively examined, all PanNECs were excluded, and 7 well-differentiated G3 PanNETs with Ki67 proliferative index lower or equal to 30% were included. Patients who underwent a macroscopically incomplete (R2) resection were not included in the study.

The prospectively collected databases of the different institutions were queried for demographic data, perioperative details, pathological findings, and follow-up data. This study was performed in accordance with the Declaration of Helsinki. Each participant institution obtained approval from the institutional review board as established by the local policy.

Surgical Procedure

All procedures were carried out by experienced surgeons in pancreatic surgery; lymphadenectomy was always performed. Conversely, splenectomy was not routinely performed during DP. However, even in spleen-preserving procedures, the splenic vessels were resected and a proper lymphadenectomy of coeliac trunk and splenic hilum was performed. Post-operative complications were categorized according to the Clavien-Dindo classification [17], and perioperative mortality was defined as surgery-related in or extra-hospital death within 90 days from surgery.

Pathological Features

In every institution, the pathological examination was performed by pathologists experienced in pancreatic malignancies and neuroendocrine neoplasms. TNM staging was defined according to the 8th UICC classification [10]. Tumour size was defined as the maximum diameter measured on the pathological specimen. Lymph node metastases were substaged into N1 (1–3 PLNs) and N2 (4 or more PLNs) according to the TNM staging proposed for pancreatic ductal adenocarcinoma and PanNEC [10]. LNR was calculated as the ratio between the number of PLNs and the total number of ELNs on the specimen and classified according to the literature [12, 18]. Resection margin status was categorized as R0

Table 1. Comparison of clinical and pathological features of patients with N0 or N+ NF-PanNETs who underwent DP

Variable	N0 (n = 209)	N+ (n = 62)	p value
Gender, *n* (%)			
Male	117 (56)	31 (50)	0.406
Female	92 (44)	31 (50)	
Age, years[a]	60 (49–66)	60 (48–66)	0.775
Resection margins, *n* (%)			
R0	204 (98)	52 (84)	**<0.001**
R1	5 (2)	10 (16)	
Ki-67 proliferative index, %[a]	2 (1–4)	5 (3–9)	**<0.001**
Grading, *n* (%)			
G1	132 (63)	13 (21)	**<0.001**
G2	73 (35)	46 (74)	
G3	4 (2)	3 (5)	
T Status, *n* (%)			
T1–T2	147 (70)	31 (50)	**0.003**
T3–T4	62 (30)	31 (50)	
Microvascular invasion, *n* (%)			
No	143 (71)	15 (25)	**<0.001**
Yes	58 (29)	46 (75)	
Perineural invasion, *n* (%)			
No	163 (85)	28 (47)	**<0.001**
Yes	29 (15)	31 (53)	
Necrosis, *n* (%)			
No	137 (91)	33 (79)	**0.031**
Yes	14 (9)	9 (21)	
Splenectomy, *n* (%)			
No	57 (27)	3 (5)	**<0.001**
Yes	152 (73)	59 (95)	
ELNs,[a]*n*	10 (4–18)	20 (13–30)	**<0.001**
ELNs, *n* (%)			
<12	122 (58)	12 (19)	**<0.001**
≥12	87 (42)	50 (81)	

Bold type denotes significance. DP, distal pancreatectomy; NF-PanNETs, non-functioning pancreatic neuroendocrine tumours; ELNs, examined lymph nodes. [a] Expressed as median (IQR).

Table 2. Multivariable linear regression analysis of factors associated with the presence of nodal metastases in 271 patients treated with DP for NF-PanNETs

Variable	*B*	95% CI	p value
Resection margins			
R0	1		**0.031**
R1	4.405	1.15–16.92	
T-stage			
T1–T2	1		0.870
T3–T4	0.870	0.33–2.29	
Grading			
G1	1		**0.029**
G2–G3	2.945	1.12–7.75	
Splenectomy			
No	1		0.055
Yes	3.962	0.97–16.16	
Microvascular invasion			
No	1		**0.043**
Yes	3.205	1.04–9.92	
Perineural invasion			
No	1		**0.048**
Yes	2.837	1.01–7.98	
Necrosis			
No	1		0.307
Yes	0.530	0.16–1.79	
ELNs[a]	1.051	1.01–1.10	**0.025**

Bold type denotes significance. ELNs, examined lymph nodes DP, distal pancreatectomy; NF-PanNETs, non-functioning pancreatic neuroendocrine tumours. [a] Expressed as continuous variable.

in case of complete tumour clearance with surgical margins microscopically uninvolved; conversely, R1 status was defined as microscopic residual tumour at resection margin. At least 3 slides from the primary tumour and all slides involving lymph nodes and vessels and their surrounding tissue were stained in haematoxylin and eosin and used to characterize microvascular invasion and perineural invasion [19–21]. Immunostaining for synaptophysin, chromogranin, and MIB1 was routinely performed. The Ki67 proliferative index was defined as the percentage of cells with MIB1-positive nuclear staining among 500–2,000 cells counted in the area of highest nuclear labelling. Tumour grade was determined according to the Ki67 proliferative index, as defined by the 2019 WHO classification [22] into G1 (Ki67 <3%), G2 (Ki67 between 3 and 20%), and G3 (Ki67 >20%).

Clinical and radiological follow-up was performed for all enrolled patients. All the patients enrolled in this study underwent a post-operative clinical and radiological follow-up. Even though the follow-up protocol was not standardized among the 4 centres, all patients underwent a high-quality imaging procedure (CT or MR), at least every year for as a minimum of 5 years from surgery. Furthermore, a ^{68}Gallium PET was routinely performed since 2012 in patients with evidence oftumour recurrence at morphological imaging.

Statistical Analysis

Continuous variables were reported as median and 25–75th interquartile range (IQR) and compared using the Mann-Whitney test. Qualitative data were reported as frequency and percentage and compared using the χ^2 test or Fisher's exact test, if appropriate.

Disease-free survival (DFS) was defined as the duration (in months) from surgery to the first evidence of disease recurrence at imaging, or it was censored at the date of last contact in case of uneventful follow-up. A minimum follow-up time of 36 months for each patient was required. Survival analyses were carried out using the Kaplan-Meier method. The potential factors associated with the risk of recurrence were explored using univariate and multivariate Cox proportional hazard models. A multivariable linear regression analysis was performed to identify predictors of PLNs. A receiver operating characteristic (ROC) curve was per-

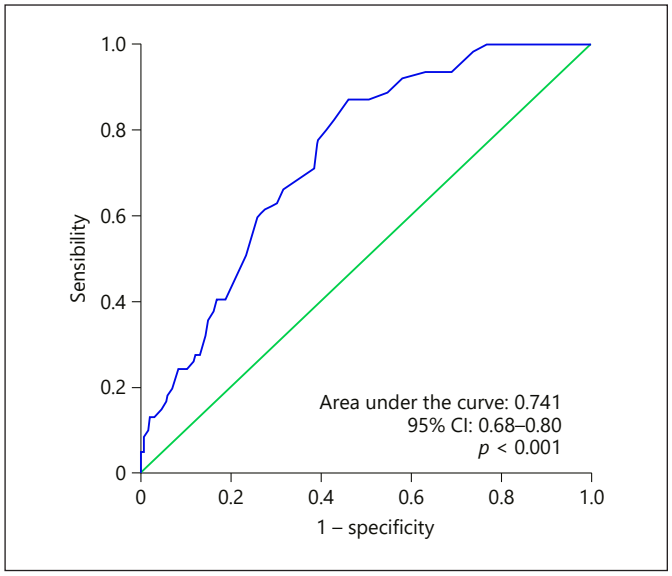

Fig. 1. ROC curve describing the sensibility and specificity of the number of ELNs in detecting the presence of PLNs. ROC, receiver operating characteristic; ELNs, examined lymph nodes; PLNs, positive lymph nodes.

formed to determine the minimum number of ELNs required to adequately predict the presence of PLNs and to enable an appropriate prognostic staging. Statistical analyses were performed using SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, USA). A 2-sided $p < 0.05$ was considered as statistically significant.

Results

Study Population

Overall, 271 patients were included. The median age at surgery was 60 (IQR 49–66) years, and 148 patients (55%) were male. The diagnosis was incidental in 153 patients (57%), while 118 patients (43%) had symptoms at diagnosis. Splenectomy was associated to DP in 211 patients (78%), and no significant differences in the rate of splenectomy were observed among the centres (data not shown). The median number of ELNs did not differ significantly among the 4 hospitals ($p = 0.160$). The median number of ELNs of the entire cohort was 12 (IQR 5–21). Patients with PanNET G3 had a higher number of ELNs (median 19 ELNs) than patients with PanNET G1 or G2 (median 11 ELNs), although this difference was not statistically significant ($p = 0.100$). A difference in terms of ELNs was also observed according to the *R* status (median of 11 ELNs [IQR 5–21] in R0 tumours and 14 ELNs [IQR 8–22] in R1 tumours, p 0.295), also in this case the

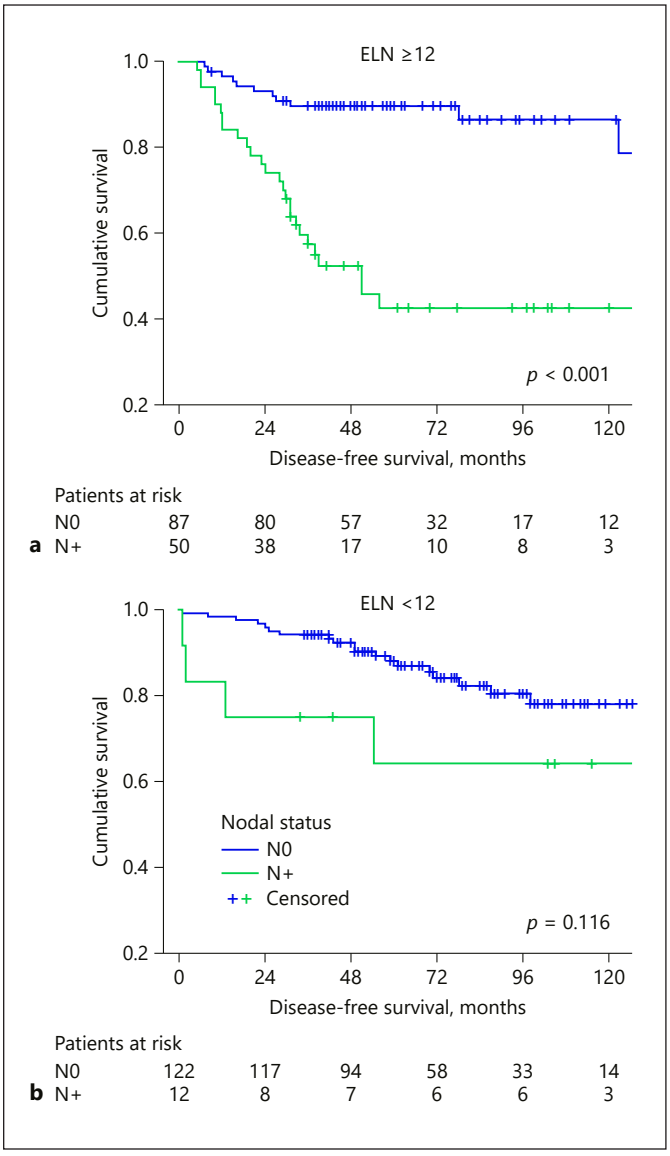

Fig. 2. Kaplan-Meier survival curves for DFS in lymph node-positive versus lymph node-negative patients who underwent DP, stratified by the number of lymph nodes retrieved. **a** Twelve or more examined lymph nodes. **b** Less than 12 examined lymph nodes. DFS, disease-free survival; DP, distal pancreatectomy.

statistical significance was not reached. Conversely, the median number of harvested nodes was significantly higher in standard DP group than that in spleen-preserving procedures (14 ELNs [IQR 8–24] vs. 7 ELNs [IQR 2–14], $p = 0.001$).

Factors Influencing the Presence of Nodal Metastases

Overall, 209 patients (77%) had N0 PanNETs, while 62 patients (23%) had at least one lymph node metastasis

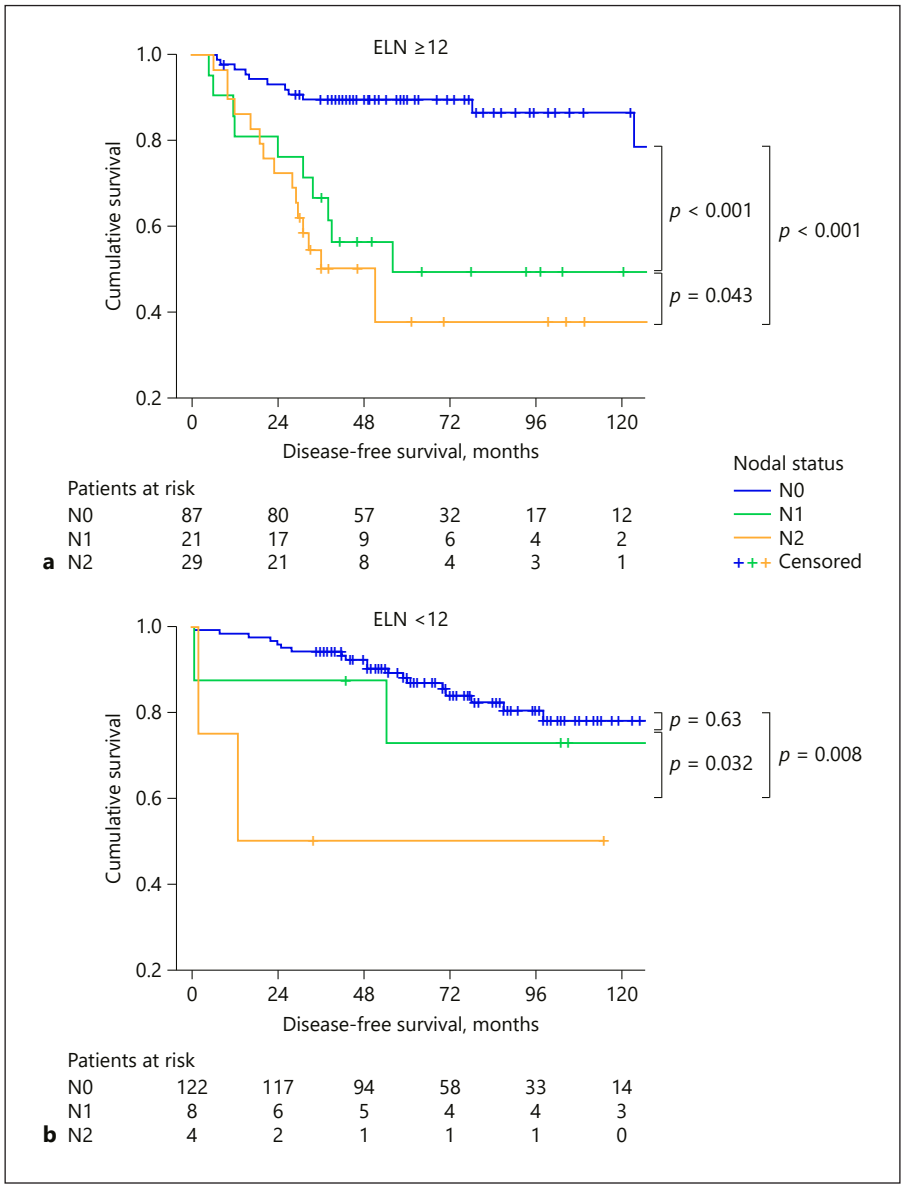

Fig. 3. Kaplan-Meier survival curves for DFS applying the N0-N1-N2 classification in patients who underwent DP, stratified by the number of lymph nodes retrieved. **a** Twelve or more examined lymph nodes. **b** Less than 12 examined lymph nodes. DFS, disease-free survival; DP, distal pancreatectomy.

(N+). The comparison of patients with or without nodal involvement is reported in Table 1. Compared to N0 tumours, N+ PanNETs were significantly more frequently staged as T3 or T4 ($p = 0.003$), were of higher grade ($p < 0.001$), had more frequent perineural ($p < 0.001$) or microvascular invasion ($p < 0.001$), positive resection margins (R1) ($p < 0.001$), and presence of necrosis ($p = 0.031$) at univariate analysis. Median Ki67 proliferative index and the number of ELNs were also significantly higher in the group of patients with N+ PanNETs. At multivariate analysis, the factors significantly independently associated with the presence of nodal metastases were higher tumour grade, R1 status, T3 or T4 stage, perineural invasion, splenectomy, and the number of ELNs (Table 2).

In order to determine the minimum number of ELNs to be resected for a correct nodal staging, a receiver operating characteristic analysis was performed (Fig. 1). 12 ELNs were the most accurate cutoff (sensitivity: 71%, specificity: 70%, area under the curve: 0.741, $p < 0.001$) in predicting the presence of PLNs. The threshold of at least 12 ELNs was also confirmed by a Kaplan-Meier analysis of the association between node-positive disease and DFS, stratified by the number of lymph nodes (LNs) retrieved during surgical resection (Fig. 2).

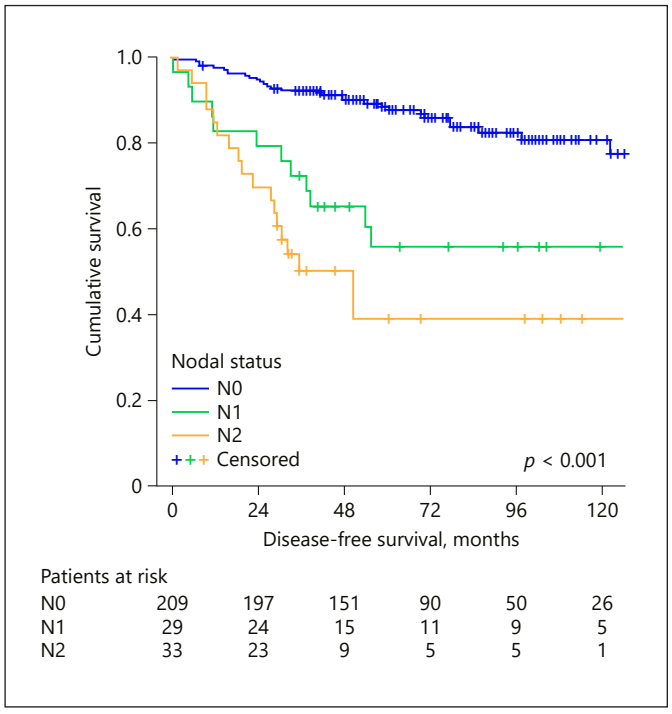

Fig. 4. Kaplan-Meier survival curve for DFS according to nodal status, applying the N0/N1/N2 classification ($p < 0.001$). DFS, disease-free survival.

For patients with ≥12 ELNs (Fig. 2a), a statistically significant difference in terms of DFS was observed between patients with N0 and N+ PanNETs ($p < 0.001$). Conversely, for patients with <12 ELNs (Fig. 2b), the N status failed to predict DFS ($p = 0.116$). Moreover, for patients with at least 12 harvested nodes, the nodal classification N0-N1-N2 maintained its prognostic value (Fig. 3a), while it was not applicable in case of <12 ELNs (Fig. 3b).

Survival Analysis

The median follow-up was 71 (IQR 44–99) months. Overall, 61 patients (22.5%) had recurrence and 6 patients (3.3%) who recurred eventually died of the disease. The median DFS for the entire cohort was 152 months, with 1- and 3-year DFS rates of 96 and 85%, respectively.

Patients with N0 PanNETs had a 3-year DFS rate of 92%, compared with 57% in patients with N+ PanNETs. When applying the N0/N1/N2 staging, the 3-year DFS rate was 72 and 50% in patients with N1 and N2 PanNETs, respectively ($p < 0.001$) (Fig. 4). Moreover, patients with an LNR = 0 had a significantly increased DFS (3-year DFS rate: 92%) compared to patients with LNR comprised between 0 and 0.40 (3-year DFS rate: 70%, $p < 0.001$) and those with LNR >0.40 (3-year DFS rate: 43%, $p < 0.001$). The univariate analysis of factors associated with DFS is shown in Table 3. On multivariate analysis, independent predictors of DFS were nodal status based on the number of PLNs (with N0 as reference; N1, HR 2.64, $p = 0.018$; N2, HR 3.66, $p = 0.001$), T stage (HR for T3 or T4: 2.87, $p = 0.006$), grade (G1, HR 1; G2-G3, HR 2.46, $p = 0.019$), and the presence of necrosis (HR 3.48, $p = 0.001$) (Table 4). Similar results were obtained when the number of PLNs was replaced by LNR (with LNR = 0 as reference; LNR [0–0.4], HR 2.16, $p = 0.033$; LNR >0.4, HR 4.86, $p = 0.007$) or by the N0/N+ status (with N0 as reference; N+, HR 2.50, $p = 0.007$) (see online suppl. Tables 1 and 2; see www.karger.com/doi/10.1159/000509709 for all online suppl. material).

Survival Sub-Analysis Excluding Well-Differentiated G3 PanNETs

Among 264 patients with G1 and G2 PanNETs, the recurrence rate was 21% (56 patients). The median DFS was 156 months, with 1- and 3-year DFS rates of 96 and 86%, respectively. The results of univariate and multivariate analysis are reported in Table 5.

Patients with N0 G1 and G2 PanNETs had a 3-year DFS rate of 93%, compared with 62% in patients with N+ G1 and G2-PanNETs. When applying the N0/N1/N2 staging, the 3-year DFS rate was 72 and 52% in patients with N1 and N2 G1–G2 PanNETs, respectively ($p < 0.001$). On multivariate analysis, independent predictors of DFS were nodal status based on the number of PLNs (with N0 as reference; N1, HR 2.52, $p = 0.036$; N2, HR 4.35, $p = 0.001$), T stage (HR for T3 or T4: 3.34, $p = 0.002$), grade (HR for G2: 2.75, $p = 0.010$), and the presence of necrosis (HR 3.14, $p = 0.003$).

Discussion

Accurate staging plays a pivotal role in oncological patients. An appropriate pathological evaluation of prognostic factors is fundamental for determining the rhythm of post-operative follow-up and the treatment strategy. This aspect is even more relevant for patients with PanNETs, given their generally prolonged survival and the lack of precise indications for adjuvant therapies or treatments in case of disease relapse [23, 24].

Many studies demonstrated that lymph node involvement is associated with a higher rate of recurrence after surgery for PanNETs [6, 25]. The present study is consistent with these results, but it also provides a new perspective on the quantitative importance of lymph node in-

Table 3. Univariate analysis of predictors of DFS in 271 patients treated with DP for NF-PanNETs

Variable	*N*	1-year DFS, %	3-year DFS, %	Median DFS, months	*p* value
Gender					
Male	148	97	88	n.r.	0.141
Female	123	93	81	n.r.	
Age					
≤60 years	144	96	85	n.r.	0.948
>60 years	127	95	86	n.r.	
Complications [17]					
None	74	91	88	n.r.	0.370
Mild complicated (CD 1–2)	140	94	82	n.r.	
Complicated (CD 3–4)	28	89	78	n.r.	
Resection margins					
R0	256	96	87	n.r.	**0.022**
R1	15	67	59	n.r.	
Grading					
G1	145	99	95	n.r.	**<0.001**
G2	119	92	75	122	
G3	7	71	43	21	
T-stage					
T1–T2	178	97	93	n.r.	**<0.001**
T3–T4	93	88	69	98	
Nodal status					
N0	209	98	92	n.r.	**<0.001**
N+	62	81	57	54	
Alternative nodal status subclassification					
N0	209	98	92	n.r.	**<0.001**
N1	29	83	72	n.r.	
N2	33	84	50	51	
ELNs					
<12	134	97	93	n.r.	**0.012**
≥12	137	94	78	152	
LNR					
0	209	98	92	n.r.	**<0.001**
>0–0.4	51	86	64	152	
>0.4	11	73	46	29	
Microvascular invasion					
No	158	98	95	n.r.	**<0.001**
Yes	104	91	69	98	
Perineural invasion					
No	191	97	91	n.r.	**<0.001**
Yes	60	88	63	70	
Necrosis					
No	170	97	88	n.r.	**<0.001**
Yes	23	78	48	26	
Splenectomy					
No	60	100	93	n.r.	**0.002**
Yes	211	94	83	n.r.	

Bold type denotes significance. CD, Clavien-Dindo classification of surgical complications [17]; ELNs, examined lymph nodes; LNR, lymph-node ratio; n.r., not reached; DFS, disease-free survival; DP, distal pancreatectomy; NF-PanNETs, non-functioning pancreatic neuroendocrine tumours.

Table 4. Multivariable analysis of predictors of DFS in 271 patients treated with DP for NF-PanNETs

Variable	*N*	HR	95% CI	*p* value
Resection margins				
R0	256	1		0.707
R1	15	0.71	0.29–2.34	
Tumour grading				
G1	145	1		**0.019**
G2–G3	126	2.46	1.16–5.23	
T-stage				
T1–T2	178	1		**0.006**
T3–T4	93	2.87	1.36–6.07	
Nodal status				
N0	209	1		
N1	29	2.64	1.75–4.17	**0.018**
N2	33	3.66	2.72–7.79	**0.001**
Microvascular invasion				
No	158	1		0.602
Yes	104	1.28	0.50–3.29	
Perineural invasion				
No	191	1		0.501
Yes	60	1.31	0.60–2.84	
Necrosis				
No	170	1		**0.001**
Yes	23	3.48	1.68–7.19	
Splenectomy				
No	60	1		0.243
Yes	211	1.77	0.68–4.58	

Bold type denotes significance. DFS, disease-free survival; DP, distal pancreatectomy; NF-PanNETs, non-functioning pancreatic neuroendocrine tumours.

volvement in resected PanNETs. The major prognostic role of lymph node metastases is already well established for pancreatic ductal adenocarcinoma. In this regard, Strobel et al. [26] found that, beyond the presence of lymph node metastases itself, the number of PLNs was an independent predictor of survival. Such evidence led, in the 8th edition of the UICC TNM staging system [10], to subclassify the N+ category into N1 (1–3 PLNs) or N2 (4 or more PLNs). The same nodal classification is currently used for PanNECs, while PanNET (G1, G2, or G3) is still classified only as N0 and N+ [10]. The present study suggests the accuracy and the prognostic relevance of the number of PLNs in the staging of PanNETs resected by DP.

The present data are consistent with the results of the study conducted by Luo et al. [27] on a large population from the SEER database, which reported that the N0/N1/N2 subclassification had a stronger prognostic impact than the N0/N1 staging. Capretti et al. [28] also reported that the number of PLNs was a better prognostic factor than the N0/N+ status. However, the follow-up time of these studies is quite limited and they are largely heterogeneous, including different types of resections and also parenchyma-sparing resections (i.e., enucleation or central pancreatectomy), which usually are not associated to an appropriate lymphadenectomy [27, 28].

For these reasons, only consecutive patients who underwent DP were included in the present study. In addition, although a high standardization of surgical procedures could not be guaranteed, a standard lymphadenectomy was always performed and pathological analysis was always conducted by dedicated pathologists. All these considerations should ensure a certain homogeneity of the cohort, thus reducing the risk of bias.

An optimal cutoff for lymph node harvesting for an appropriate nodal staging following DP performed for NF-PanNET was proposed in the present study. Regarding ductal adenocarcinoma, a minimum number of 12–15 resected nodes are usually considered appropriate for nodal staging [29]. As an example, Lahat et al. [30] reported a different DFS for a cutoff of 13 ELNs among patients with N0 neoplasms. In patients with ductal adenocarcinoma, the number of resected nodes is correlated with the probability of finding lymph node metastases [26]. A similar result was identified in our study, with every additional resected lymph node giving an additional probability of detecting a nodal metastasis of 5%.

Regarding pancreaticoduodenectomy for PanNETs, Partelli et al. [12] described that at least 13 lymph nodes should be yielded for accurate nodal staging. A similar result regarding DP is herein reported, as a significant difference, in term of DFS, between the number of patients with N0 and N+ neoplasms was observed in patients with at least 12 ELNs, whereas the N status failed to predict disease relapse in case of <12 ELNs.

PanNETs are generally considered indolent neoplasms compared to their exocrine counterpart, and this assumption may lead to a bias in surgical strategy and management. Patients with PanNET are often treated by atypical resections (i.e., enucleation or central pancreatectomy) without a formal lymphadenectomy or with just a limited nodal sampling [31]. For this reason, preoperative evaluation may play a role in identifying patients at high risk of nodal involvement. In our series, Ki67 index and T-stage confirmed their association with the presence of nodal metastases. For patients preoperatively judged at high risk of nodal involvement, a formal resection with a proper lymphadenectomy is mandatory.

Table 5. Univariate and multivariate analysis of predictors of DFS considering only 264 G1 and G2 patients treated with DP for NF-PanNETs

Variable	*N*	Univariate analysis				Multivariate analysis		
		1-year DFS, %	3-year DFS, %	Median DFS, months	*p* value	H.R.	95% CI	*p* value
Gender								
Male	143	93	91	n.r.	0.074			
Female	121	93	82	n.r.				
Age								
≤60 years	140	97	86	n.r.	0.771			
>60 years	124	93	86	n.r.				
Complications [17]								
None	73	95	90	n.r.	0.476			
Mild complicated (CD 1–2)	135	96	84	n.r.				
Complicated (CD 3–4)	27	93	81	n.r.				
Resection margins								
R0	251	96	87	n.r.	0.227			
R1	13	77	68	n.r.				
Grading								
G1	145	98	95	n.r.	**<0.001**	1		
G2	119	92	75	123		2.75	1.26–5.95	**0.010**
T-stage								
T1–T2	177	98	94	n.r.	**<0.001**	1		
T3–T4	87	90	71	152		3.34	1.54–7.24	**0.002**
Nodal status[a]								
N0	205	98	93	n.r.	**<0.001**			
N+	59	90	62	56				
Alternative nodal status subclassification								
N0	205	98	93	n.r.	**<0.001**	1		
N1	29	90	72	n.r.		2.52	1.62–3.76	**0.036**
N2	30	90	52	51		4.35	2.76–10.72	**0.001**
ELNs								
<12	132	97	92	n.r.	**0.028**	1		
≥12	132	95	81	n.r.		0.98	0.45–2.17	0.967
LNR[a]								
0	205	98	93	n.r.	**<0.001**			
>0–0.4	50	92	68	152				
>0.4	9	78	44	29				
Microvascular invasion								
No	152	98	95	n.r.	**<0.001**	1		
Yes	98	93	72	152		0.87	0.33–2.29	0.777
Perineural invasion								
No	189	97	91	n.r.	**<0.001**	1		
Yes	55	91	67	23		1.65	0.75–3.62	0.210
Necrosis								
No	168	96	88	n.r.	**<0.001**	1		
Yes	19	79	53	49		3.14	1.46–6.76	**0.003**
Splenectomy								
No	60	98	93	n.r.	**0.005**	1		
Yes	204	95	85	n.r.		1.99	0.74–5.37	0.175

Bold type denotes significance. CD, Clavien-Dindo classification of surgical complications [17]; ELNs, examined lymph nodes; LNR, lymph-node ratio; n.r., not reached; DFS, disease-free survival; DP, distal pancreatectomy; NF-PanNETs, non-functioning pancreatic neuroendocrine tumours. [a] Nodal status and LNR were not included in the multivariate model because of collinearity with the alternative nodal status subclassification.

The number of ELNs seems to influence somehow the long-term outcomes; indeed, the LNR resulted as one of the most powerful predictors of DFS. Boninsegna et al. [32] reported the prognostic role of LNR in a series of 57 patients with pancreatic neuroendocrine neoplasms (PanNENs). However, another multicentric study [9] did not report a difference in terms of DFS based on the value of LNR. This result may be biased by the presence, in this latter report, of atypical resections, while in the present study only DP was considered.

This study has several limitations, mainly related to its retrospective nature. Despite the high surgical experience in the 4 centres, the uniformity in surgical resections and lymphadenectomies is not completely guaranteed. In particular, the extent of lymphadenectomy may be influenced by the presence of aggressiveness features known before surgery, such as G3 PanNET. Nevertheless, the similar median number of ELNs observed among the 4 institutions should guarantee a fair level of homogeneity. The problem of standardization may be applied also to pathological examination, even though all 4 hospitals are high-volume centres with strong experience in PanNENs. Given the pivotal importance of nodal staging in determining the prognosis, pathological examination should be extremely accurate, performed by experienced pathologists, and highly standardized, as for ductal adenocarcinoma [33], in order to examine the highest number of resected lymph node.

Another limitation of the present study is the different median number of ELNs between spleen-preserving procedures and standard DP. These findings suggest that spleen-preserving procedures should be recommended only in selected cases without features of biological aggressiveness. However, also in spleen-preserving procedures, a proper lymphadenectomy of coeliac trunk and splenic hilum should be performed in order to harvest an adequate number of ELNs. In particular, in a randomized clinical trial on gastric cancer patients, Yu et al. [34] reported that the same number of LNs was harvested both in spleen-preserving procedures and in those including splenectomy. This result suggests that a proper lymphadenectomy of the splenic hilum could be reached even preserving the spleen.

A further limitation is the lack of information regarding nodal metastases localization on the histopathological report. A precise nodal metastases mapping should be indispensable, particularly to discriminate peripancreatic PLNs versus distant PLNs. In small bowel NENs, a recent study [35] demonstrated the possibility of skip nodal metastases. So far, this event was never demonstrated for PanNENs, otherwise it might influence substantially the pattern and the frequency of nodal involvement. However, only a prospective study including a standard protocol for lymphadenectomy, with a precise nodal mapping, and a standardized pathological examination may overcome all these limitations.

In conclusion, a minimal number of 12 nodes should be harvested in case of DP for NF-PanNET for an appropriate nodal staging. The number of positive lymph nodes is an independent predictor of DFS survival after DP for NF-PanNET, and the N0/N1/N2 nodal classification seems to be more relevant than the current N0/N+ staging.

Statement of Ethics

The study was conducted in ethical accordance with the World Medical Association Declaration of Helsinki. All the patients signed a written informed consent for the anonymous utilization of their clinical data. In Italy, no ethical approval was required due to the retrospective nature of the study. In France, an institutional review board approval for data collection (IRB 00006477, Paris 7 University, France) was obtained.

Conflict of Interest Statement

The authors declare no conflict of interest.

Funding Sources

Valentina Andreasi's PhD scholarship was financed by the Gioja Bianca Costanza Found, a private, no-profit foundation, without any conflict of interest in the present study.

Author Contributions

The authorships were assigned in accordance to the ICMJE criteria for authorship: Giovanni Guarneri, Louis De Mestier, and Alain Sauvanet contributed substantially to the conception and design of the work, to the analysis and interpretation of the data, in drafting the work, in its revision, and final approval. Valentina Andreasi, Luca Landoni, Stefano Partelli, Chiara Nessi, Michele Fontana, Safi Dokmak, Bertrand Dousset, and Philippe Ruszniewski contributed to the design of the work, to data collection, in drafting the work, and in its final revision. Sébastien Gaujoux, Claudio Bassi, and Massimo Falconi contributed in the conception of the work, in the interpretation of data, and in the final revision and critical correction of the manuscript.

References

1 Milan SA, Yeo CJ. Neuroendocrine tumors of the pancreas. Curr Opin Oncol. 2012 Jan; 24(1):46–55.
2 Dasari A, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol. 2017;3(10):1335–42.
3 Taal BG, Visser O. Epidemiology of neuroendocrine tumours. Neuroendocrinology. 2004; 80(Suppl 1):3–7.
4 Falconi M, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, et al. ENETS consensus guidelines update for the management of patients with functional pancreatic neuroendocrine tumors and non-functional pancreatic neuroendocrine tumors. Neuroendocrinology. 2016;103(2):153–71.
5 Ellison TA, Wolfgang CL, Shi C, Cameron JL, Murakami P, Mun LJ, et al. A single institution's 26-year experience with nonfunctional pancreatic neuroendocrine tumors: a validation of current staging systems and a new prognostic nomogram. Ann Surg. 2014; 259(2):204–12.
6 Hashim YM, Trinkaus KM, Linehan DC, Strasberg SS, Fields RC, Cao D, et al. Regional lymphadenectomy is indicated in the surgical treatment of Pancreatic Neuroendocrine Tumors (PNETs). Ann Surg. 2014;259(2):197–203.
7 Genç CG, Jilesen AP, Partelli S, Falconi M, Muffatti F, Van Kemenade FJ, et al. A new scoring system to predict recurrent disease in grade 1 and 2 nonfunctional pancreatic neuroendocrine tumors. Ann Surg. 2018;267(6): 1148–54.
8 Partelli S, Gaujoux S, Boninsegna L, Cherif R, Crippa S, Couvelard A, et al. Pattern and clinical predictors of lymph node involvement in nonfunctioning pancreatic neuroendocrine tumors (NF-PanNETs). JAMA Surg. 2013 Oct;148(10):932–9.
9 Partelli S, Gaujoux S, Boninsegna L, Cherif R, Crippa S, Couvelard A, et al. Pattern and clinical predictors of lymph node involvement in nonfunctioning Pancreatic Neuroendocrine Tumors (NF-PanNETs). JAMA Surg. 2013 Oct;148(10):932–9.
10 Brierley JD, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours. 8th ed.; 2017.
11 Zhang P, Li YL, Qiu XD, Luo J, Shi YF, Sun YL, et al. Clinicopathological characteristics and risk factors for recurrence of well-differentiated pancreatic neuroendocrine tumors after radical surgery: a case-control study. World J Surg Oncol. 2019 Apr 11;17(1):66.
12 Partelli S, Javed AA, Andreasi V, He J, Muffatti F, Weiss MJ, et al. The number of positive nodes accurately predicts recurrence after pancreaticoduodenectomy for nonfunctioning neuroendocrine neoplasms. Eur J Surg Oncol. 2018 Jun;44(6):778–83.
13 Bettini R, Boninsegna L, Mantovani W, Capelli P, Bassi C, Pederzoli P, et al. Prognostic factors at diagnosis and value of WHO classification in a mono-institutional series of 180 non-functioning pancreatic endocrine tumours. Ann Oncol. 2008 May;19(5):903–8.
14 Tomassetti P, Campana D, Piscitelli L, Casadei R, Santini D, Nori F, et al. Endocrine pancreatic tumors: factors correlated with survival. Ann Oncol. 2005 Nov;16(11):1806–10.
15 Fischer L, Kleeff J, Esposito I, Hinz U, Zimmermann A, Friess H, et al. Clinical outcome and long-term survival in 118 consecutive patients with neuroendocrine tumours of the pancreas. Br J Surg. 2008 May;95(5): 627–35.
16 Lopez-Aguiar AG, Zaidi M, Dillhoff M, Beal EW, Poultsides GA, Makris E, et al. Defining the role of lymphadenectomy for pancreatic neuroendocrine tumors: an eight institution study of 695 patients from the U.S. Neuroendocrine Tumor Study Group. J Clin Oncol. 2018 Aug;26(8):2517–24.
17 Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004 Aug;240(2):205–13.
18 Liu P, Zhang X, Shang Y, Lu L, Cao F, Sun M, et al. Lymph node ratio, but not the total number of examined lymph nodes or lymph node metastasis, is a predictor of overall survival for pancreatic neuroendocrine neoplasms after surgical resection. Oncotarget. 2017 Jul 12; 8(51):89245–55.
19 Ceyhan GO, Liebl F, Maak M, Schuster T, Becker K, Langer R, et al. The severity of neural invasion is a crucial prognostic factor in rectal cancer independent of neoadjuvant radiochemotherapy. Ann Surg. 2010 Nov; 252(5):797–804.
20 Liebl F, Demir IE, Rosenberg R, Boldis A, Yildiz E, Kujundzic K, et al. The severity of neural invasion is associated with shortened survival in colon cancer. Clin Cancer Res. 2013 Jan 1;19(1):50–61.
21 Panaro F, Kellil T, Vendrell J, Sega V, Souche R, Piardi T, et al. Microvascular invasion is a major prognostic factor after pancreatico-duodenectomy for adenocarcinoma. J Surg Oncol. 2019 Sep;120(3):483–93.
22 IARC Publications. WHO classification of tumours. Digestive system tumours. 5th ed. Lyon: 2019.
23 Ricci C, Casadei R, Taffurelli G, Campana D, Ambrosini V, Pacilio CA, et al. Is radical surgery always curative in pancreatic neuroendocrine tumors? a cure model survival analysis. Pancreatology. 2018 Apr;18(3):313–7.
24 Genç CG, Falconi M, Partelli S, Muffatti F, van Eeden S, Doglioni C, et al. Recurrence of pancreatic neuroendocrine tumors and survival predicted by Ki67. Ann Surg Oncol. 2018 Aug;25(8):2467–74.
25 Landoni L, Marchegiani G, Pollini T, Cingarlini S, D'Onofrio M, Capelli P, et al. The evolution of surgical strategies for pancreatic neuroendocrine tumors (Pan-NENs). Ann Surg. 2019 Apr;269(4):725–32.
26 Strobel O, Hinz U, Gluth A, Hank T, Hackert T, Bergmann F, et al. Pancreatic adenocarcinoma: number of positive nodes allows to distinguish several N categories. Ann Surg. 2015 May;261(5):961–9.
27 Luo G, Jin K, Cheng H, Guo M, Lu Y, Wang Z, et al. Revised nodal stage for pancreatic neuroendocrine tumors. Pancreatology. 2017 Jul–Aug;17(4):599–604.
28 Capretti G, Nappo G, Smiroldo V, Cereda M, Branciforte B, Spaggiari P, et al. The number of metastatic lymph nodes is a useful predictive factor for recurrence after surgery for nonmetastatic nonfunctional neuroendocrine neoplasm of the pancreas. Gastroenterol Res Pract. 2019 Apr 21;2019:6856329.
29 Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. Cancer J Clin. 2017 Mar; 67(2):93–9.
30 Lahat G, Lubezky N, Gerstenhaber F, Nizri E, Gysi M, Rozenek M, et al. Number of evaluated lymph nodes and positive lymph nodes, lymph node ratio, and log odds evaluation in early-stage pancreatic ductal adenocarcinoma: numerology or valid indicators of patient outcome? World J Surg Oncol. 2016 Sep 29; 14(1):254.
31 Parekh JR, Wang SC, Bergsland EK, Venook AP, Warren RS, Kim GE, et al. Lymph node sampling rates and predictors of nodal metastasis in pancreatic neuroendocrine tumor resections: the UCSF experience with 149 patients. Pancreas. 2012 Aug;41(6):840–4.
32 Boninsegna L, Panzuto F, Partelli S, Capelli P, Delle Fave G, Bettini R, et al. Malignant pancreatic neuroendocrine tumour: lymph node ratio and Ki67 are predictors of recurrence after curative resections. Eur J Cancer. 2012 Jul; 48(11):1608–15.
33 Capella C, Albarello L, Capelli P, Sessa F, Zamboni G. Carcinoma of the exocrine pancreas: the histology report. Dig Liver Dis. 2011 Mar;43(Suppl 4):S282–92.
34 Yu W, Choi GS, Chung HY. Randomized clinical trial of splenectomy versus splenic preservation in patients with proximal gastric cancer. Br J Surg. 2006 May;93(5):559–63.
35 Pasquer A, Walter T, Rousset P, Hervieu V, Forestier J, Lombard-Bohas C, et al. Lymphadenectomy during small bowel neuroendocrine tumor surgery: the concept of skip metastases. Ann Surg Oncol. 2016 Dec;23(Suppl 5):804–8.