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Neuroendocrine tumors of the pancreas: a retrospective single-center analysis using the ENETS TNM-classification and immunohistochemical markers for risk stratification

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Abstract

Background: This study was performed to assess the 2006 introduced ENETS TNM-classification with respect to patient survival and surgical approach for patients who underwent surgery for a neuroendocrine tumor of the pancreas (PNET).

Methods: Between 2001 and 2010 38 patients after resection of a PNET were investigated regarding tumor localization and size. Further, patient survival with regards to the new TNM-classification, the operation methods and immunohistochemical markers was analyzed.

Results: The estimated mean survival time of the 38 patients was 91 ± 10 months (female 116 ± 9, male 56 ± 14 months; p = 0.008). The 5-year survival rate was 63.9%. Patient survival differed significantly depending on tumor size (pT1 107 ± 13, pT2 94 ± 16, pT3 44 ± 7 and pT4 18 ± 14 months; P = 0.006). Patients without lymph node metastasis survived significantly longer compared to patients with positive lymph node status (108 ± 9 vs. 19 ± 5 months; P < 0.001). However, survival in patients with and without distant metastasis did not differ significantly (92 ± 11 vs. 80 ± 23 months; P = 0.876). Further, the tumor grading significantly influenced patient survival (G1 111 ± 12, G2 68 ± 12 and G3 21 ± 14 months; P = 0.037).

Conclusions: As part of the TNM-classification especially lymph node status and also tumor size and grading were identified as important factors determining patient survival. Further, gender was demonstrated to significantly influence survival time. If an R0 resection was achieved in patients with distant metastases patient survival was comparable to patients without metastasis.

Keywords: Neuroendocrine Tumors Of The Pancreas, Tnm Classification, Risk Stratification, Lymph Node Metastasis, E-Cadherin, B-Catenin, Cyclin D1, Il-17a

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Background

Neuroendocrine tumors represent a heterogenous group of neoplasms regarding biological features and clinical behavior [1-3]. These tumors are rare with an incidence between 3.24 and 6.5 per 100,000 [4]. However, over the last years there has been a remarkable increase in frequency with predominant localization of the neuroendocrine tumors in the gastrointestinal tract or the bronchopulmonary system [4]. Approximately 5% of all neuroendocrine tumors develop in the pancreas with one of the poorest outcomes (35% 5-year survival rate) [3,5-8]. The WHO classification published in 2000 and modified by Kloeppel at al. in 2004 distinguishes between well-differentiated neuroendocrine tumors, well-differentiated neuroendocrine carcinomas and poorly-differentiated neuroendocrine carcinomas [7]. Because of the wide variety in clinical behavior between these tumor entities, a tailored surgical and medical therapy based on this classification was challenging [9]. A new TNM classification with a grading system based on the mitotic count and the Ki-67 index was established by the European Neuroendocrine Tumor Society (ENETS) to address this problem in 2006. In this system PNETs with a size <2 cm are classified as T1, with a size from 2–4 cm as T2, with >4 cm or invasion of duodenum or bile duct as T3, and all tumors invading adjacent organs or the wall of large vessels as T4 [10]. The status of regional lymph nodes and distant metastasis were taken into account analogously to the TNM classification system of other tumors in combination with a grading system according to mitotic count and the Ki-67-index. Based on these criteria, a risk stratification of patients using different disease stages was proposed. Stage I includes pT1 tumors, stage IIa pT2 tumors, stage IIb pT3 tumors and stage IIIa pT4 tumors all without lymph node or distant metastasis. Tumors of any pT stage with lymph node metastasis are classified in stage IIIb and all tumors with distant metastasis are characterized as stage IV [10]. In 2010 the UICC/AJCC/WHO 2010 TNM staging system was introduced which differs significantly from the 2006 ENETS TNM staging. Due to the fact that Rindi et al. demonstrated that the 2006 ENETS TNM staging system is superior to the UICC/AJCC/WHO 2010 TNM staging system we decided to use the 2006 ENETS TNM staging for our study [11]. Since the 2006 ENETS TNM classification is based on the published experience of single centers and not on a uniform database, the purpose of this study was to analyze neuroendocrine tumors of the pancreas that were resected in our center with regards to clinicopathological characteristics that influence patient survival. Further, we wanted to assess the new 2006 ENETS TNM classification system and its prognostic value for long-term survival.

Additionally to Ki-67 that as marker for proliferation is included in the TNM classification systems it has been shown previously that pancreatic endocrine neoplasms and adenocarcinoma of the pancreas express E-cadherin and β-catenin, both being important for cell-cell contact, and that cyclin D1 and IL-17A contribute to malignant transformation in pancreatic tumors [12-17]. In our study we tested if different expression levels of these markers influence patient survival.

Methods

Study setting

The study was performed at the University Medical Center of Regensburg, Germany in the Department of Surgery in compliance with the Helsinki Declaration and was approved by the ethics committee of the University Medical Center Regensburg, Germany (Nr. 13-180-0248).

Study cohort

Patients with PNETs confirmed by histology who underwent surgery at our center between 2001 and 2010 were identified using the hospital computer data base (access available with permission of the University Medical Center Regensburg, Germany). Only patients with a surgically possible R0 resection were included (N = 38). If a distant metastasis was present these metastases were resected simultaneously which was achieved in 6 out of the 7 patients with distant metastases. General patient information like age and gender were documented. Tumor localization was categorized as pancreas head, corpus or tail based on intraoperative findings and histology. Partial pancreatoduodenectomy (Kausch-Whipple operation), pancreas tail resection with and without splenectomy and localized resection were used as operation methods, individually adapted for tumor size, localization and extent of tumor infiltration into other organs. In case of a localized resection a systematic lymphadenectomy was only performed in case of suspicious lymph nodes in the preoperative diagnostic. Survival data were obtained using the database of the regional tumor center of East Bavaria, Germany (access freely available for hospitals in the region of East Bavaria, Germany). Two patients were lost to follow-up and were excluded from the survival analysis.

Histology

The specimens were fixed in 4% paraformaldehyde and embedded in paraffin. The tumor tissue sections were reevaluated by a pathologist based on the 2006 ENETS TNM classification [10]. Additionally, tissue sections from the tumor region were also stained for endocrine markers and the proliferation marker Ki-67 and a mitotic count was conducted for each specimen. Therefore, positive nuclear staining (MIB-1 antibody) in 10 subsequent high power fields in areas with highest proliferative activity was counted. Further, immunohistochemistry of tissue microarray sections was performed. 2 μm tissue microarray

sections were first deparaffinized and then automated stained for cyclin D1 (RM-9104-R7, NeoMarkers Inc., Fremont, CA, USA; Dilution 1:25), E-cadherin (M3612, Dako North America Inc., Carpinteria, CA, USA; Dilution 1:50), β-catenin (sc-7963, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; Dilution 1:50) and IL-17A (AF-317-NA, R&D Systems Inc. Minneapolis, MN, USA; Dilution 1:50) with a Ventana BenchMark Ultra IHC/ISH Staining Module (Ventana Medical Systems, Inc., Tucson, Arizona, USA) according to the manufacturers staining procedures. Stained tissue microarrays were analyzed by an independent pathologist blinded for patient and survival data. E-cadherin and β-catenin expression was quantified in 3 levels as low, intermediate and high, cyclin D1 and IL-17A expression was described in 2 levels as low or high.

Statistics

For statistical analysis SPSS 18.0 for Windows (Copyright SPSS Inc., Chicago, Illinois, USA) was used. The distribution of age at operation, follow-up period, tumor size and operation time was described as mean ± standard deviation and compared using Mann–Whitney-*U*-test due to the fact that these values do not show a normal distribution. Survival after surgery was estimated using the Kaplan-Meier method. The log rank test was used to compare survival stratified according to histological classification and grading, tumor localization, operation method, age at operation, gender and different immunohistochemical groups. Distributions of tumor stages, nodal status, metastasis, grading and operation methods were analyzed with Pearsons's chi squared test. P values < 0.05 were considered statistically significant.

Results

General characteristics

In total 38 patients (18 men, 20 women) with PNETs were included in this retrospective analysis. The mean follow-up time was 45 months. Among the 38 PNETs, twelve neoplasms (31.6%) were localized in the pancreas head, eight (21.1%) in the pancreas corpus and eighteen (47.4%) in the pancreas tail. Eight patients (21.1%) had a functional (3 glucagonomas, 5 insulinomas) and thirty patients (78.9%) a non-functional PNET. Two patients were lost during follow-up and were excluded from survival analysis. Out of the 36 persons who completed follow-up, ten (27.8%) died during this period. The mean age at operation did not significantly differ between women and men (54 ± 16 vs. 50 ± 16 years). The mean tumor size was 3.5 ± 0.5 cm (range 0.3 – 15.0 cm; women 3.2 ± 3.6 vs. 3.7 ± 2.8 cm) (Table 1).

Table 1 General characteristics of patients with PNETs

	Female	Male	p
N	20(52.6%)	18(47.4%)	
Age at operation (years)	54±16	50±16	0.400
Tumor size (cm)	3.2±3.6	3.7±2.8	0.596
Tumor localization			0.621
Pancreas head	5	7	
Pancreas corpus	5	3	
Pancreas tail	10	8	
pT Stage			0.164
pT1	8	5	
pT2	8	4	
pT3	2	7	
pT4	1	2	
Nodal Status			0.095
NO	17	11	
N1	3	7	
Metastasis			0.024
cMO	19	12	*
cM1	1	6	*
Grading			0.023
G1	13	5	*
G2	6	10	*
G3	0	3	*

Age and tumor size presented as mean±SD.Group comparisons using the Mann-White-*U*-test or persons's chi-squared test (*p<0.05).

TNM classification

According to the TNM classification, 13 of the PNETs were staged as pT1 (34%), 12 as pT2 (32%), 9 as pT3 (24%) and 3 as pT4 (8%) with no statistically significant difference in gender distribution. In one patient there was not enough material left for a histological reevaluation of the tumor. Therefore, this patient was excluded from the analysis regarding T stages and grading. 28 of the patients (74%) had a negative lymph node status whereas in 10 patients (26%) the lymph nodes were infiltrated by tumor cells. Though there was a trend towards increased lymph node infiltration in male patient the level of significance was not reached. Distant metastases were present in seven patients (18%) with a statistically significant higher ratio of men (P = 0.024), in the remaining 31 patients (82%) no metastasis were found. Eighteen of the 37 PNETs were graded as G1 (47%), 16 as G2 (42%) and 3 as G3 (8%) tumors with a statistically significant higher tumor grading in male patients (P = 0.023). (Additional file 1: Table S1; detailed TNM staging of individual patients in Additional file 1: Table S1).

Operation methods

Eight patients (21%) were treated with a localized resection either as enucleation of the tumor or as pancreas segment resection. Five patients (13%) received a spleen

preserving pancreas tail resection, fourteen patients (37%) had a pancreas tail resection with splenectomy and in eleven patients (29%) a partial pancreatoduodenectomy (Kausch-Whipple operation) was performed. For a tumor localization in the pancreas head only in 1 out of 12 patients (8%) a localized resection was used compared to a higher rate of a limited surgical approach in the pancreas corpus (3 out of 8 localized resections; 38%) and in the pancreas tail (4 out of 18 localized resections (22%) and 5 out of 18 (28%) spleen preserving pancreas tail resections.

Gender but not tumor localization influences patient survival

Survival curves were calculated using the Kaplan-Meier method. The mean overall survival time was 91 ± 10 months and the 5-year overall survival rate was 64%. Women had an estimated mean survival time of 116 ± 9 months which was significantly longer compared to men with 56 ± 14 months (P = 0.008) (Figure 1).

The mean survival time for PNETs localized in the pancreas head was 49 ± 9 months, in the pancreas corpus 70 ± 18 months and in the pancreas tail 105 ± 12 months without significant differences.

T stage, nodal status and grading but not distant metastasis influence survival of resected patients

Further, the influence of the tumor size on patient survival after resection of the PNETs was assessed. In this analysis pT1 tumors showed a mean survival of 107 ± 13 months, pT2 tumors of 94 ± 16 months, pT3 tumors of 44 ± 7 months and pT4 tumors of 18 ± 14 months. Survival of pT1, pT2 and pT3 stages was statistically significant different to survival of patients with pT4 tumors (pT1 vs. pT4 P = 0.001; pT2 vs. pT4 P = 0.026; pT3 vs. pT4 P = 0.047) (Figure 2A).

Additionally, the influence of the lymph node status on patient survival was tested using the Kaplan-Meier method. Patients with negative lymph node status had a statistically significant longer estimated mean survival time of 108 ± 9 months compared to patients with tumor infiltrated lymph nodes with an estimated mean survival time of 19 ± 5 months (P < 0.001) (Figure 2B).

Furthermore, a survival analysis with regards to distant metastasis was performed. Interestingly, mean survival time of cM1 tumors (N = 7) was not significantly different compared to cM0 tumors (N = 31) (80 ± 23 months vs. 92 ± 11 months; P = 0.876) (Figure 3A). Notably, within the patients that developed distant metastases the distribution of tumor grading (2 G1, 4 G2, 1 G3) was

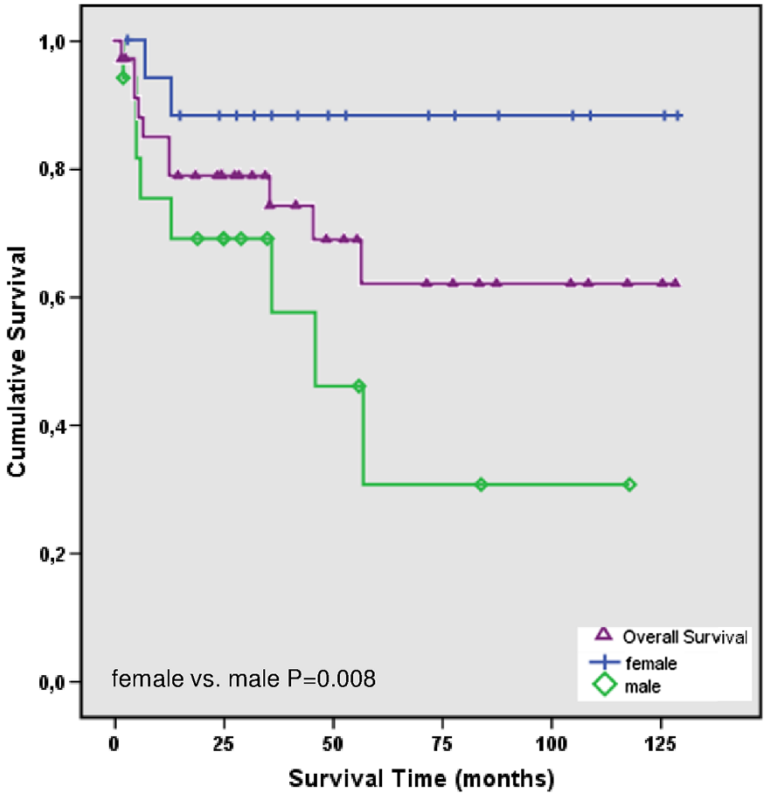

Figure 1 Overall survival and survival stratified for gender. The mean overall survival time was 92 ± 10 months and the 5-year overall survival rate was 63.9%. Women showed a significantly longer mean survival time compared to men (116 ± 9 versus 56 ± 14 months; P = 0.008).

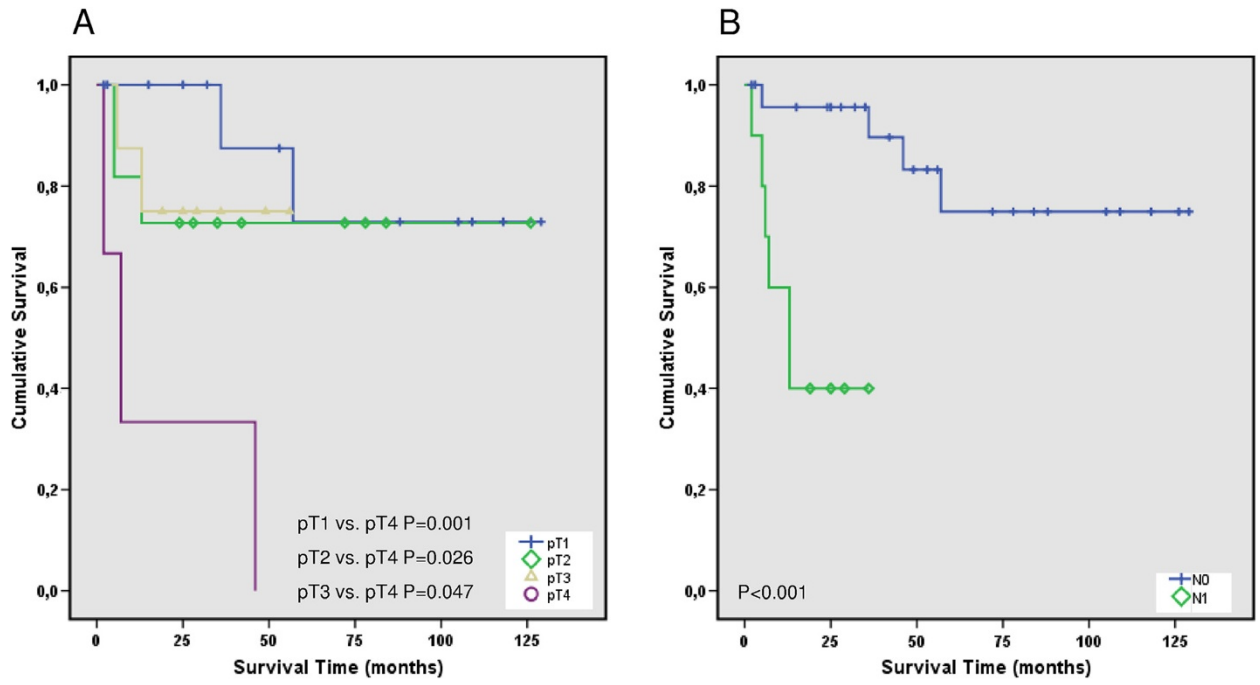

Figure 2 Patient survival stratified for pT stages and lymph node status. **(A)** The survival of pT1 tumors (N = 13; 107 ± 13 months), pT2 tumors (N = 12; 94 ± 16 months), pT3 tumors (N = 9; 44 ± 7 months) and pT4 tumors (N = 3; 18 ± 14 months) differed significantly (pT1 vs. pT2 P = 0.485; pT1 vs. pT3 P = 0.266; pT1 vs. pT4 P = 0.001; pT2 vs. pT3 P = 0.862; pT2 vs. pT4 P = 0.026; pT3 vs. pT4 P = 0.047). **(B)** N0 (N = 28) tumors showed significant longer survival time compared to N1 (N = 10) tumors (108 ± 9 versus 19 ± 5 months; P < 0.001).

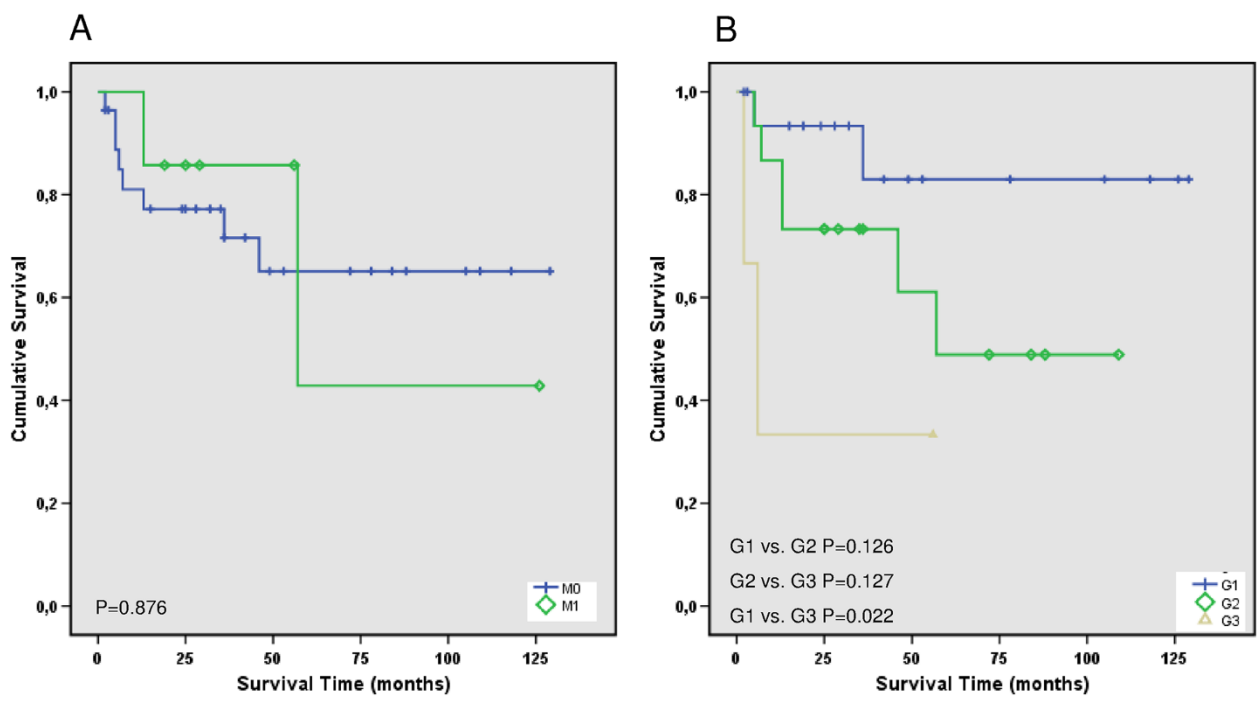

Figure 3 Patient survival stratified for distant metastasis and grading. **(A)** Survival time of patients without metastasis (N = 31) was statistically not different to patients with distant metastases (N = 7) (92 ± 11 versus 80 ± 23 months; P = 0.876). **(B)** The survival of patients with G1 tumors (N = 18; 111 ± 12 months), G2 tumors (N = 16; 68 ± 12 months) and G3 tumors (N = 3; 21 ± 14 months) differed significantly (G1 vs. G2 P = 0.126; G1 vs. G3 P = 0.022; G2 vs. G3 P = 0.127).

not different to patients without distant metastases (16 G1, 12 G2, 2 G3; P = 0.471).

Next, the influence of tumor grading on patient survival was investigated. Patients with a PNET histologically classified as G1 had a mean survival time of 111 ± 12 months, compared to 68 ± 12 months for patients with a G2 tumor and 21 ± 14 months when the tumor was classified as G3. The difference in patient survival between G1 and G3 tumors was statistically significant (G1 vs. G2 P = 0.126; G1 vs. G3 P = 0.022; G2 vs. G3 P = 0.127) (Figure 3B).

Finally, survival analysis using the disease staging proposed by the new TNM classification revealed that patients with a stage IIIb PNET showed significantly shorter survival (12 ± 5 months; P < 0.001) compared to patients in stage I, IIa, IIb, IIIa and IV (I 117.38 ± 10.87; IIa 74.12 ± 9.24; IIb 49.00 ± 0; IIIa 46.00 ± 0 months; IV 80.30 ± 23.35 months). Between stage I, IIa, IIb, IIIa and IV no significant difference was found (Figure 4).

Intratumoral expression of cell adhesion proteins E-cadherin and β-catenin and expression of cyclin D1 and IL-17A do not influence patient survival

To test if E-cadherin, β-catenin, cyclin D1 and IL-17A influence patient survival, these markers were additionally used to stain the PNET tissue samples. Kaplan-Meier analysis was performed for the respective immunohistochemical staining.

Low (67 ± 21 months), intermediate (91 ± 16 months) and high intratumoral expression levels of the adhesion protein E-cadherin (82 ± 16 months) did not show significant differences in patient survival (P = 0.855) (Figure 5A). Also, low (70 ± 18 months), intermediate (97 ± 16 months) and high intratumoral expression levels of β-catenin (77 ± 16 months) revealed no significant differences in patient survival (P = 0.795) (Figure 5B).

Patient survival with low (86 ± 11 months) and high (92 ± 15 months) expression levels of Cyclin D1 in the tumors was not significantly different (P = 0.795) (Figure 6A). Further, the analysis of patients with low intratumoral expression levels of IL-17A (87 ± 14 months) showed a trend to reduced survival times compared to patients with high intratumoral IL-17A expression (117 ± 11 months) though the difference was not statistically significant (P = 0.211) (Figure 6B).

Discussion

In this study the lymph node status was detected to be an important and statistically significant factor for patient

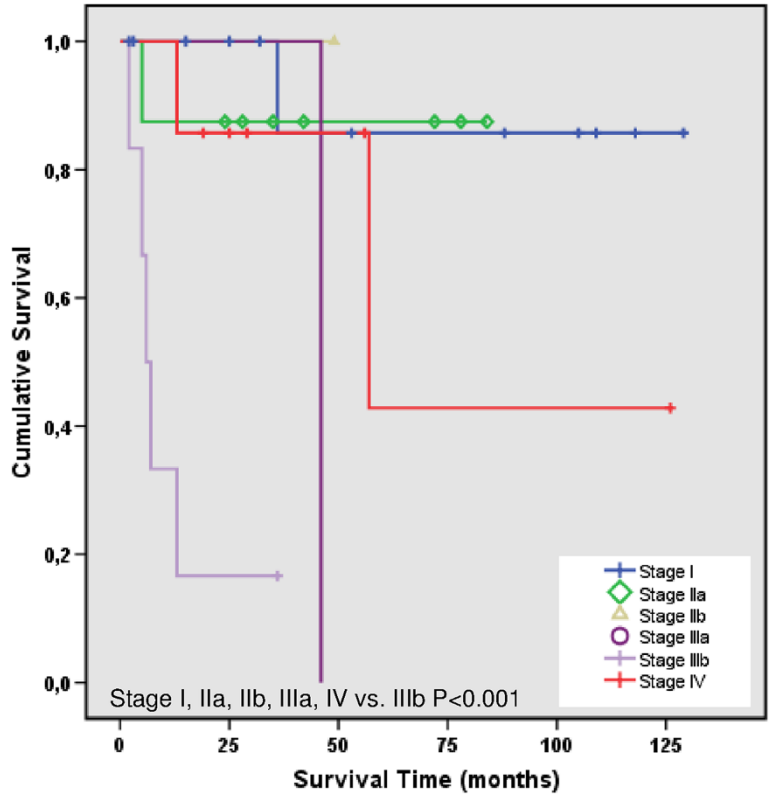

Figure 4 Patient survival stratified for disease stages. Patients with stage IIIb disease showed significant shorter survival (12 ± 5 months; P < 0.001) compared to patients in stage I (117 ± 11 months), IIa (74 ± 9 months), IIb (49 ± 0 months), IIIa (46 ± 0 months) and IV (80 ± 23 months). Between stage I, IIa, IIb, IIIa and IV no statistically significant difference could be found.

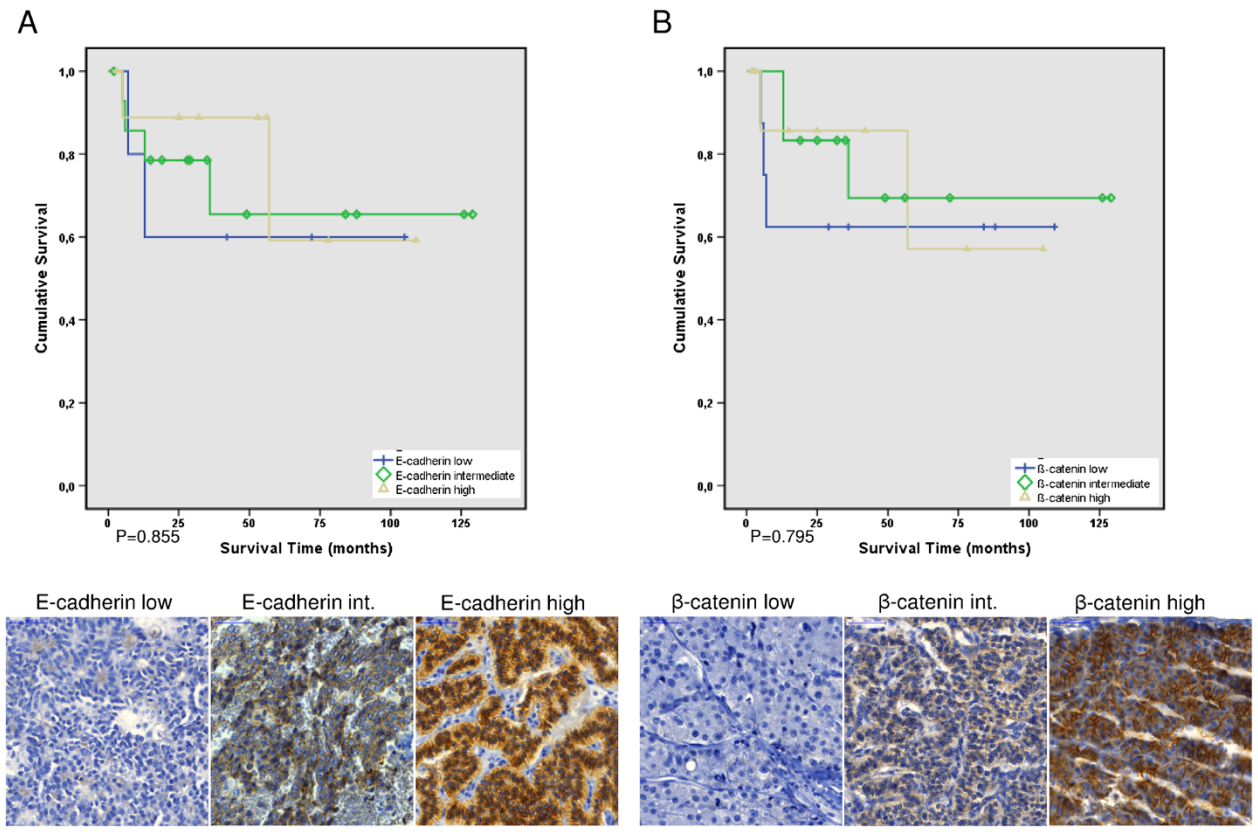

Figure 5 Patient survival stratified for intratumoral E-cadherin and β-catenin expression. **(A)** There was no statistically significant survival difference between patients with low (N = 9), intermediate (N = 15) and high (N = 13) expression levels of E-cadherin (67 ± 21 versus 91 ± 16 versus 82 ± 16 months; P = 0.855). **(B)** No statistically significant survival differences were noted for patients with low (N = 12), intermediate (N = 13) and high (N = 12) expression levels of β-catenin (70 ± 18 versus 97 ± 16 versus 77 ± 16 months; P = 0.795).

survival after resection of a PNET with a mean survival time of 108 ± 9 months for N0 in contrast to 19 ± 5 months for N1 tumors (P < 0.001). This is supported by reports from Ito and Scarpa et al. who both also identified lymph node metastasis as relevant prognostic factor [9,18]. However, a large study with 3851 patients by Bilimoria et al. using the National Cancer Data Base found the nodal status not to be associated with survival [19]. A possible explanation for these contrary findings may be the use of different surgical approaches in these studies and also the rather long time of patient recruitment between 1985 and 2004 in the epidemiologic study by Bilimoria et al. Similar to previously published results, in this study the pT staging and grading provided a statistically significant prognostic stratification of patients [9,18,20].

Different to other tumor entities, in our study distant metastasis had no impact on patient survival. This is supported by the study of Fischer et al. who also found no statistical influence of distant metastasis on survival [2]. However, Scarpa et al. observed distant metastasis to be a statistically significant factor for survival [9]. These contrary findings may be explained by very diverging patient cohorts and low number of cases in our study, which is one limitation of this study additionally to the fact that data were analyzed retrospectively at a single-center. However, these critical result of our study can be explained since in the study by Scarpa et al. also palliative patients without resection were included whereas in ours and Fischer's study only patients with surgical resection of the tumor were analyzed [2,9]. Taken together, these results suggest that distant metastasis may have no impact on patient survival if they are properly resected.

As a consequence of the findings that positive lymph nodes but not distant metastasis influence survival of resected patients, we were not able to get a good survival stratification using the proposed 2006 ENETS TNM stages which stands in contrast to other studies [10,18]. Only patients suffering from stage IIIb tumors showed significantly worse survival in our analysis. Also the modified staging system proposed by Scarpa et al. would not result in a better risk stratification in our patient cohort [9]. However, this most probable is due to low case numbers in our study since a large study by Rindi et al. demonstrated that this ENETS TNM stages

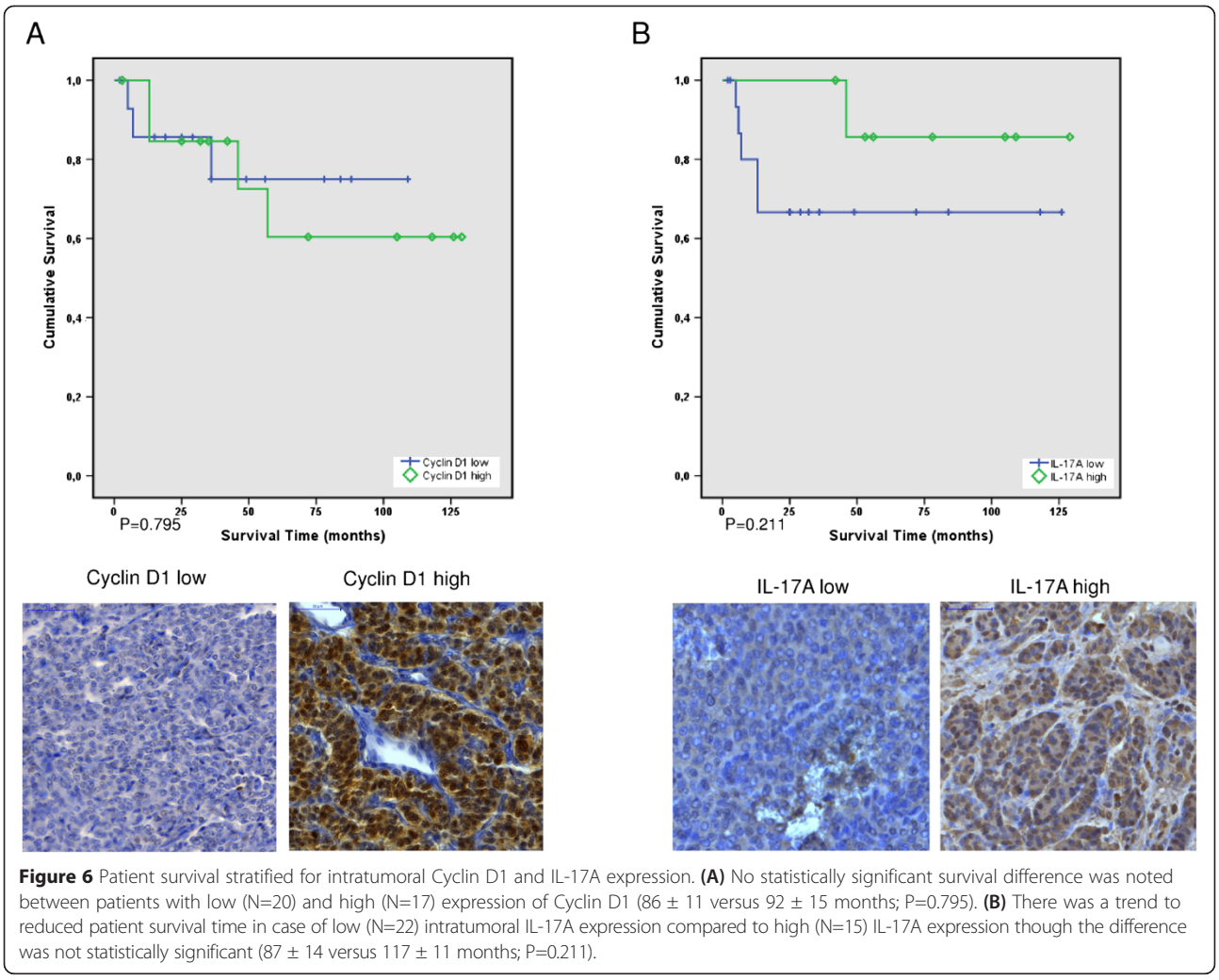

Figure 6 Patient survival stratified for intratumoral Cyclin D1 and IL-17A expression. **(A)** No statistically significant survival difference was noted between patients with low (N=20) and high (N=17) expression of Cyclin D1 (86 ± 11 versus 92 ± 15 months; P=0.795). **(B)** There was a trend to reduced patient survival time in case of low (N=22) intratumoral IL-17A expression compared to high (N=15) IL-17A expression though the difference was not statistically significant (87 ± 14 versus 117 ± 11 months; P=0.211).

work well and are even superior to the UICC/AJCC/WHO 2010 TNM staging system [11].

Very interesting was the fact, that we found statistically significant differences in survival between women and men (116 ± 9 months vs. 56 ± 14 months; P = 0.008). This is supported by a recent study from Kim et al. who found gender to be a prognostic factor for disease free survival of neuroendocrine tumors and also by Rindi et al. who also shows male patients being at higher risk [11,21]. One reason for shorter survival of male patients may be the trend to more tumor positive lymph nodes and the higher tumor grading in our patient cohort, however, these differences between men and women were not statistically significant and therefore both groups were comparable. Possible explanations for these differences in tumor biology and survival may be gender imbalances for risk factors like smoking and alcohol, a different constitution or hormonal influences as suggested by Kim et al. [21].

Since previous studies demonstrated that pancreatic endocrine neoplasms and adenocarcinoma of the pancreas express E-cadherin and β-catenin and that further cyclin D1 and IL-17A contribute to malignant transformation in pancreatic tumors, these markers were used to stain PNET tissue samples [12-17]. Cell adhesion proteins like E-cadherin and β-catenin have also been shown to promote tumor progression and are associated with poor patient survival in colorectal adenocarcinoma and in a subgroup of patients with non-small cell lung cancer if expressed at low levels [22-25]. However, no influence on patient survival was found in our PNET cohort. Further, no correlation of Cyclin D1 with patient survival was shown in the investigated PNETs in contrast to other reports with different tumor entities before [26,27]. In the analysis of intratumoral IL-17A expression a trend towards reduced patient survival in case of low IL-17A expression was noted, however, the differences were not statistically significant. So far, there have been conflicting

reports about the effects of IL-17A expression in tumor microenvironment on survival [28-31]. In our cohort of PNET patients the patient number might be too small to conclude if intratumoral IL-17A expression is beneficial. Also, the other tested factors were not suitable for patient risk stratification.

The surgical approach in this study was based on the individual preoperative diagnostic results with the goal to achieve an R0 resection. Limited tumors with regards to TNM staging and grading were treated with a localized resection or a pancreas tail resection without splenectomy. If distant metastasis were present, a simultaneous resection was performed. This operation strategy is supported by reports from Hodul et al. and others [3,32,33]. According to published results, we found an overall 5-year survival rate of 63.9% [9,18,19].

Taking into account that we detected the lymph node status and the tumor grading to be the essential histopathological prognostic factors for survival of patients with surgically resected PNETs it seems to be very important to identify these patients at high risk before surgery. For this purpose, the ^{68}Ga-DOTATOC positron emission tomography/computed tomography may emerge as a useful tool not only for the evaluation of the primary tumor and the distant metastasis but also for the detection of lymph node metastasis as suggested by Kumar et al. [34]. Then an individualized surgical therapy could be performed. If an extensive lymph node dissection with the goal of achieving an R0 situation or a neoadjuvant therapy concept with ^{99}Y-Dotatoc as suggested by Stoelzing et al. can improve the poor outcome of these patients needs to be evaluated in future studies [34,35].

Conclusion

In conclusion, we identified the lymph node status and also tumor size and grading as important survival factors within the new ENET classification system but not distant metastasis if an R0 resection was achieved. This is important for a tailored therapy of patients with PNETs.

Additional file

Additional file 1: Table S1. Detailed TNM staging patients.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SMB designed the study concept, collected and analyzed data, and wrote the manuscript. FW and JMW collected and analyzed data. SAF, AA and HJS analyzed data and reviewed the manuscript. MH designed the study concept, collected and analyzed data, and reviewed the manuscript. All authors read and approved the final manuscript.

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