Prognostic factors of long-term outcome in gastroenteropancreatic neuroendocrine tumours

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Abstract

Neuroendocrine tumours (NET) of the gastroenteropancreatic system comprise a malignant entity with a low incidence. Only limited information is available on long-term clinical outcome and clinically applicable prognostic factors. We performed a retrospective analysis of a large, well-characterized centre-based patient cohort of 399 patients with histologically proven NET. Data were analysed according to epidemiological, clinical and histopathological characteristics. Detailed survival analyses using the Kaplan–Meier method were performed. Prognostic factors were tested by log-rank testing and independent risk factors were analysed using a Cox regression model. In the studied cohort, primary tumours originated in the fore-, mid- and hindgut in 46.1, 37.1 and 4.5% respectively. Extra-intestinal or unknown primary tumours were present in 8.4 and 10.5% respectively. Distant metastasis was present at initial diagnosis in 69.4%. Most frequent metastatic sites were liver (85%), peritoneal cavity (18%), bones (8%), other intra-abdominal sites (6%) and lungs (4%). Overall, 5- and 10-year survival rates were 78 and 63% respectively. Time to progression after initial diagnosis was significantly shorter in pancreatic as compared with ileal NET. Survival analysis revealed significantly better clinical outcome for primary tumours smaller than 25 mm, absence of metastasis, absence of any clinical symptoms, positive immunohistochemical staining for chromogranin A and a lower Ki67 index. These results were confirmed as independent by multivariate analysis. Therefore, this large retrospective analysis of a well-documented cohort of patients with NET demonstrates several prognostic factors of clinical relevance and wide availability, which should be considered for risk stratification in the management of NET.

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Introduction

Neuroendocrine tumours (NET) are relatively infrequent and mostly slowly growing human neoplasms with an estimated annual incidence of 1–2 per 100 000 (Modlin *et al*. 2003). These tumours are related to the endocrine cells of the diffuse endocrine system (DES) of the human body (Rindi *et al*. 2004) and therefore occur in many different primary tumour localizations. For one hundred years, the term 'carcinoid tumour', which was introduced by Oberndorfer (1907), has been widely used for these neoplasms, although it is not precise and several attempts for a more accurate terminology have been made. In 2000, the WHO suggested the term (neuro-) endocrine tumour or

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carcinoma in a recent classification system (Solcia *et al*. 2000, Klöppel *et al*. 2004). The most important cell biological features of NET, which they share with normal endocrine cells of the DES, are interaction with chromium or silver salts (i.e. enterochromaffinity and argentaffinity; Gosset & Masson 1914), electron microscopic evidence of hormone-containing large dense-core vesicles (Pearse 1969) or immunohistochemical positivity for vesicle proteins such as chromogranin A (Lloyd & Wilson 1983) or synaptophysin (Wiedenmann *et al*. 1986). The last two are now used for routine immunohistochemical diagnosis of NET together with conventional haematoxylin–eosin staining and labelling of the Ki67 protein to estimate the proliferating cell fraction (Solcia *et al*. 2000, Klöppel *et al*. 2004, Rindi *et al*. 2004).

In 1963, Williams & Sandler attempted a first clinicopathological classification according to embryonic origin of the presumed endocrine precursor cells. They differentiated foregut (i.e. bronchial, gastric, duodenal, pancreatic) from mid- (i.e. jejunal, ileal, appendiceal, caecal, ascending and right transverse colonic) and hindgut NET (i.e. left transverse colonic to rectal). This classification proved to be of only limited value because it is too inaccurate (Klöppel *et al*. 2004). According to the largest series of NET studied to date (Modlin *et al*. 2003), the majority of NET arise within the gastroenteropancreatic system (GEP) while the bronchial tract represents the second most frequent primary tumour localization. However, metastasized NET with an undetectable primary tumour have been noted in more than 4% of all cases in several studies (Janson *et al*. 1997, Kirshbom *et al*. 1998, Shebani *et al*. 1999, Levi *et al*. 2000, Quaedvlieg *et al*. 2001, Modlin *et al*. 2003, Pape *et al*. 2004, Soga 2005).

Although histopathological diagnosis and classification of NET have improved considerably over the last decades, only few data are available on clinical outcome of large and well-characterized patient cohorts. Several factors have contributed to this: first, only few population- (Levi *et al*. 2000, Hemminki & Li 2001, Quaedvlieg *et al*. 2001, Modlin *et al*. 2003) or centre-based (Janson *et al*. 1997, Kirshbom *et al*. 1998, Shebani *et al*. 1999, Onaitis *et al*. 2000, Van Gompel *et al*. 2004, Pape *et al*. 2004, Panzuto *et al*. 2005) epidemiological studies have been published and only very limited data are available from other types of studies such as diagnostic studies or interventional trials. Second, due to the various clinicopathological classifications that have been used for NET throughout the decades (Williams & Sandler 1963, Capella *et al*. 1995, Solcia *et al*. 2000, Klöppel *et al*. 2004) the diagnosis of a neoplasm with endocrine features has been made with varying precision. Therefore, comparable information on relevant prognostic factors is scarce and frequently disparate. Third, many terms and specifics, which are used for characterization of NET, have been used incoherently and therefore comparisons between published data are extremely difficult. Fourth, data from databases, from clinical centres and from pathological units confer differing information. Details depend on inclusion criteria into a database, selection criteria for admission to clinical centres and referral specifics to pathology units. All of these data must be interpreted in the context of their specific background.

Thus, we have retrospectively studied data from our centre to overcome some of these difficulties. Although a specific centre-based bias has to be taken into account by this approach, this strategy has the advantage of providing a well-characterized homogenous study population in which comparable sets of data can be acquired for each patient. Most of these information were recorded on regular routine patient visits in a prospective manner but were amended where necessary and possible in a retrospective fashion. The WHO classification of (neuro-) endocrine tumours of 2000 (Solcia *et al*. 2000) was readily established in our department and therefore provided the basis for clinicopathological evaluation of NET and future management. We are therefore able to present survival analysis and statistical evaluation of prognostic factors in a large, well-characterized patient cohort of NET patients with special consideration of recent clinicopathological classification systems.

Materials and methods

A retrospective analysis was performed based on the medical records of 399 patients treated at our department between 1980 and 2004. Patients were included in the analysis only if the histopathological diagnosis of NET disease had been confirmed and documented. A systematic review of patient files was performed and epidemiological information as well as tumour specific information were recorded and reviewed for correctness and consistency. These included information on date of initial diagnosis, observation period, death, date of death and cause of death if available, date of histopathological diagnosis, clinical information on symptoms, functionality, results of imaging and surgical procedures, time to first progression, localization of the primary tumour, extent of metastasis if present, treatments and clinical outcome.

Special attention was paid to histopathological data that were acquired from the diagnosing institution. When the diagnosing institution was external the pathological diagnosis was reviewed and revised when appropriate by a staff pathologist from our institution (M K). Particularly, histological differentiation grade, immunohistochemistry and the Ki67-labelling index were amended when sufficient tumour tissue was available. For classification, the clinicopathological classification by Capella *et al.* (1995) and the WHO classification (Solcia *et al.* 2000) for histological typing of endocrine tumours were applied whenever possible based on the available information.

Statistical analysis was performed using SPSS software version 11.0 (SPSS Inc., Chicago, IL, USA) and SAS version 8.02 (SAS Institute, Cary, NC, USA). When approximately normally distributed, data are given as mean $\pm$ S.E.M., otherwise median values and full ranges are given. Survival analysis was performed using the Kaplan–Meier method and log-rank testing for univariate analysis of potentially prognostic factors. For multivariate testing of several independent risk factors for NET-associated death, a Cox regression model was used after adjustment for age and gender. $P < 0.05$ was considered statistically significant.

Results

General characterization of the patient cohort

Data from 399 patients with histologically proven NET were analysed. Mean age at initial diagnosis was 54 years (range 7–85 years), gender distribution was almost even (female/male ratio 0.93; 48.1/51.9%), median follow-up was 25 months (range 0–268 months). The diagnosis was made at an external institution or hospital in 67.4% (269/399) and our own institution in the remaining patients. NET disease was hereditary (i.e. associated with multiple endocrine neoplasia type-1 syndrome; MEN-1) in only 1.4% of all cases. A second malignant neoplasm was diagnosed (either prior to or after NET diagnosis) in 7.1% (28/295 cases with available information), the most frequent being prostate cancer (nine cases), renal cell carcinoma (four cases), breast cancer (three cases) and colorectal cancer (three cases).

During the observation period, 24% (97/399) of the patients died. In 82.5% (80/97) of the cases, the cause of death could be defined. In 70.1% (68/80) of the cases, the cause of death was associated with NET disease (i.e. tumour cachexia, liver failure due to NET metastases, respiratory failure due to NET metastases, septic syndrome due to malignant ascites). Estimated median survival for NET-related death according to the Kaplan–Meier analysis was 169 months in the whole cohort of 399 patients with cumulative 5- and 10-year survival rates of 78 and 63% respectively (see Fig. 1 and Table 1). During the observation period and for survival analysis purposes censored at the time of last visit to our department, 145 patients were lost to follow-up. Data on the clinical course after initial diagnosis were available in 295 cases. In these cases, tumour progression occurred with a median time to progression of 17 months (range 1–211 months) after initial diagnosis.

Primary tumour characteristics

Primary tumour localization is detailed in Table 1. NET of the stomach included 20 cases of ECLomas (type-I gastric NET), 11 sporadic low-grade malignant NET (type III) and 4 high-grade malignant NET. In 10.5% of all cases, a primary tumour could not be localized albeit extensive diagnostic efforts. Gender distribution varied slightly between the most frequent NET primary tumour localizations (refer to Table 1). The estimated 5- and 10-year-survival-rates (YSR) according to the Kaplan–Meier analysis for the most frequent primary tumour localizations are also given in Table 1. In type-I gastric NET, the estimated 5-YSR was 100% while it was 80% in type III gastric NET ($P = 0.0514$). Of the four patients with high-grade malignant gastric NET no one survived for more than

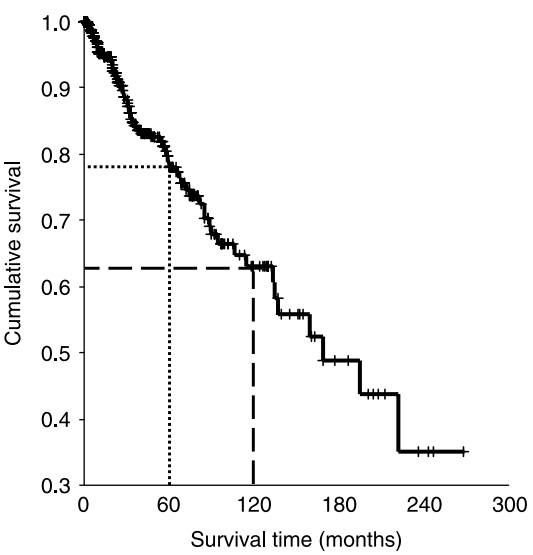

Figure 1 Cumulative survival analysis according to the Kaplan–Meier method of 399 NET (+, censored cases) including 5- (dotted line) and 10- (dashed line) year survival rates.

Table 1 Distribution of neuroendocrine tumours (NET) according to primary tumour localization including gender distribution and estimated 5- and 10-year-survival-rates (YSR) according to the Kaplan–Meier analysis for the most frequent primary tumour localizations

According to embryonal origin	Anatomical localization	Frequency (numbers)	Frequency (% of cases)	Gender distribution (female/male)	5-YSR	10-YSR
Foregut $n=184$ (46.1%)	Thymus	3	0.8	–	–	–
	Bronchopulmonary	23	5.8	–	–	–
	Oesophagus	3	0.8	–	–	–
	Stomach	38	9.5	47.4/52.6%	85%	56%
	Duodenum	18	4.5	–	–	–
	Pancreas	98	24.6	44.9/55.1%	69%	62%
	Liver	1	0.3	–	–	–
Midgut $n=148$ (37.1%)	Jejunum	11	2.8	–	–	–
	Ileum	104	26.1	50/50%	90%	63%
	Appendix	22	5.5	88.2/31.8%	–	–
	Caecum	10	2.5	–	–	–
	Colon, ascending	1	0.3	–	–	–
Hindgut $n=18$ (4.5%)	Colon, sigmoid	1	0.3	–	–	–
	Rectum	17	4.2	–	–	–
Extraintestinal $n=7$ (1.8%)	Ovaries	3	0.8	–	–	–
	Others	4	1	–	–	–
Unknown primary $n=42$ (10.5%)		42	10.5	–	74%	55%
Total		399	100	48.1/51.9%	78%	63%

10 months, which was significantly different from type I and III gastric NET ($P=0.0027$ and 0.0339 respectively).

Mean age at initial diagnosis was 54 years (range 24–81 years) in pancreatic NET and 57 years (range 10–83 years) in ileal NET. Although estimated 5- and 10-YSR differed for pancreatic and ileal NET, overall cumulative survival showed only a tendency towards significantly longer survival in ileal NET ($n=202$; $P=0.0730$). However, median time to first progression was significantly shorter (analysed in 164 cases; $P=0.0089$ by univariate analysis) in pancreatic NET ($n=78$; median: 12 months, range 1–128 months) as compared with ileal NET ($n=86$; median: 22.5 months, range 1–211 months; Fig. 2).

Size of the primary tumour at initial diagnosis was studied when the information was available (270/399). Median size of the primary tumour was 2.5 cm (range 0.2–1.7 cm); pancreatic primaries were generally larger (median 3.5 cm, range 0.9–1.5 cm) than ileal primaries (median 2.5 cm, range 0.4–10 cm). By univariate analysis, a primary tumour size of <2.5 cm was associated with a significantly better outcome than a primary tumour >2.5 cm ($P=0.0006$; Fig. 3).

Role of metastasis

At initial diagnosis metastasis was present in 86.2% (332/385) of all cases with the available information. Distant metastasis was observed in 71.9% (277/385) while sole loco-regional lymph node metastasis was found in 14.3% (55/385). The site of distant metastasis at initial diagnosis (data from 190 patients) and during the course of the disease (171 patients) is presented in Table 2. Metastasis in relation to primary tumour localization was analysed in 253 cases (Fig. 4). The incidence rate of distant metastasis for the two most

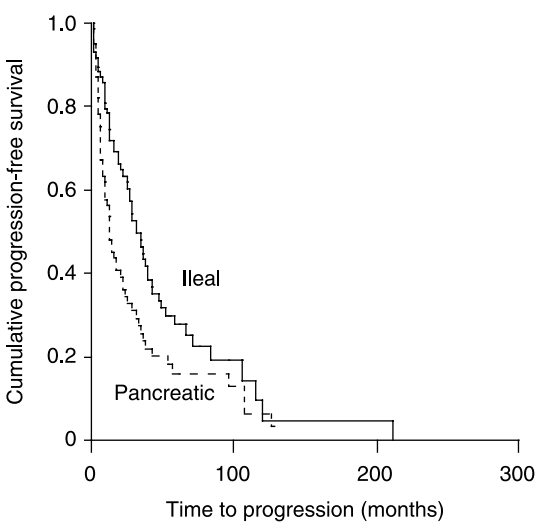

Figure 2 Cumulative progression-free survival of pancreatic (broken line) compared with ileal (solid line) NET ($P=0.0089$).

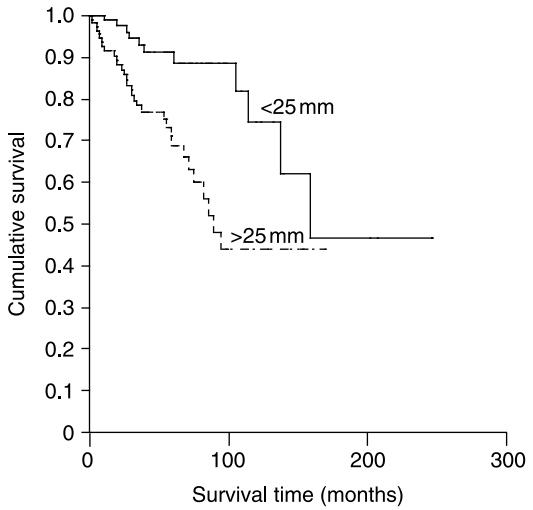

Figure 3 Cumulative survival of NET according to a size of <25 mm (solid line) as compared with >25 mm (broken line) of the primary tumour ($P=0.0006$).

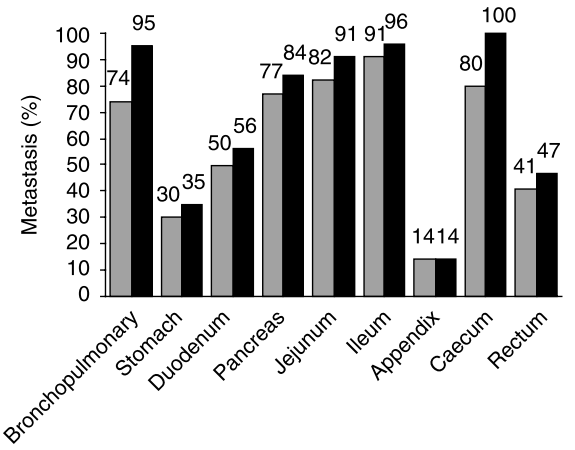

Figure 4 Occurrence of metastasis (per cent of cases) in 253 NET with available information according to primary tumour localization at initial diagnosis (grey bars) and during the course of the disease (black bars).

frequent primary tumours was 77% (pancreatic) and 91% (ileal) at initial diagnosis and increased during the course of the disease to 84 and 96% respectively. Presence of metastasis at initial diagnosis was associated with a significantly poorer outcome ($P=0.0024$; Fig. 5).

Role of clinical symptoms and functionality

Clinical symptoms at initial diagnosis were evaluated in 83.9% (335/399). Clinical symptoms were present in 87.2% (292/335) while incidental NET diagnosis was made without any symptoms in 12.8% (43/335). Symptoms were classified as either non-specific in 69.5% or specific with symptoms from hormone hypersecretion of NET (i.e. functionality) in 30.5% (see Table 3). Functionality was diagnosed during the course of the disease in 33 additional patients, so that a total of 36.4% (122/335) of patients suffered from functionality during the course of NET disease. The most frequent functional syndrome was carcinoid syndrome present in 95 out of 335 patients as detailed in Table 3. A carcinoid syndrome was also present at initial diagnosis in 12 out of 42 cases (28.6%) of NET with unknown primary tumour. Carcinoid heart disease with fibrotic alterations of the right heart was diagnosed by echocardiography in 19.6% (18/92) of patients with carcinoid syndrome necessitating cardiac surgery for right heart failure in three patients (16.7%).

While the absence of clinical symptoms, whether specific or non-specific, was associated with a significantly better outcome ($P=0.026$), this could

Table 2 Sites and frequency of distant metastasis at initial diagnosis and during the course of the disease

Localization of metastases	**Frequency at initial diagnosis** (numbers (% of 190 cases))	**Frequency during course of the disease** (numbers (% of 171 cases))
Liver	161 (84.7%)	146 (85.4%)
Peritoneum	35 (18.4%)	35 (20.5%)
Bones	16 (8.4%)	27 (15.8%)
Lungs	7 (3.7%)	22 (12.9%)
Brain	2 (1.1%)	5 (2.9%)
Other extraabdominal	2 (1.1%)	–

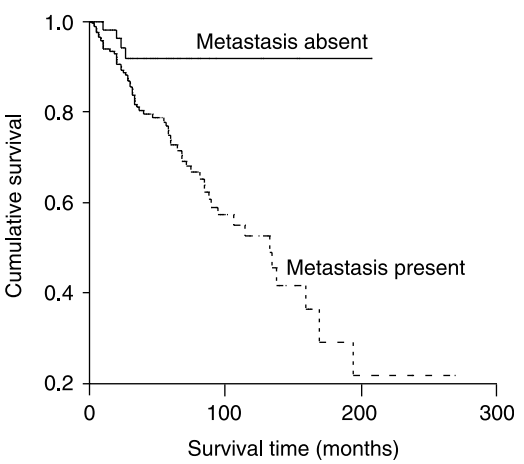

Figure 5 Cumulative survival of NET according to absence (solid line) or presence (broken line) of metastasis at initial diagnosis ($P=0.0024$).

Table 3 Symptoms in 355 neuroendocrine tumours (NET) patients

Non-specific symptoms at initial diagnosis	Frequency (numbers (% of cases))
(a) Types and frequency of non-specific symptoms	
Total	203/292 (69.5%)
Abdominal pain	160/203 (78.8%)
Weight loss	74/203 (36%)
Small bowel obstruction	36/203 (17.7%)
Fatigue	34/203 (16.7%)
GI bleeding	21/203 (10.3%)
Night sweats	16/203 (7.9%)
Jaundice	9/203 (4.4%)
Fever of unknown origin	5/203 (2.5%)
Palpable tumour mass	5/203 (2.5%)
Functional syndromes at initial diagnosis	**Frequency (numbers (% of cases))**
(b) Specific hormone hypersecretion-related functional syndromes at initial diagnosis	
Total	89/292 (30.5%)
Carcinoid syndrome	63/89 (70.8%)
Flush	52/63 (82.5%)
Diarrhoea	46/63 (73%)
Bronchospasm	4/63 (6.3%)
Carcinoid heart disease	12/63 (19%)
Zollinger–Ellison's syndrome	15/89 (16.9%)
Whipple's triad	8/89 (9%)
Verner–Morrison syndrome	2/89 (2.2%)
Glucagonoma syndrome	1/89 (1.1%)
Primary tumour localization in carcinoid syndrome	**Frequency (numbers (% of cases))**
(c) Distribution of primary tumour localization in patients with carcinoid syndrome at initial diagnosis	
Total	95/335 (28.4%)
Foregut	20/95 (21.1%)
Pancreas	11/20 (55%)
Bronchopulmonary	5/20 (25%)
Duodenum	2/20 (10%)
Stomach	1/20 (5%)
Thymus	1/20 (5%)
Midgut	62/95 (65.3%)
Ileum	53/62 (85.5%)
Jejunum	4/62 (6.5%)
Caecum	3/62 (4.8%)
Appendix	2/62 (3.2%)

not be demonstrated for the presence or absence of a specific functional syndrome versus non-specific symptoms.

Influence of histopathological classification

Although histological diagnosis had been made in all patients, details on histological features such as cell size, nuclear morphology, angio- and neuroinvasion were available only in a limited number of cases. Histopathological diagnosis was made from the primary tumour (biopsy or surgical specimen) in 273 cases, from biopsy of liver metastases in 53 and from other metastases in 43 cases.

Neuroendocrine differentiation was substantiated in all cases by immunohistochemical positivity for either chromogranin A (90.8%, 268/295) or synaptophysin (100%, 253/253) or both. In the chromogranin A-negative cases synaptophysin staining confirmed neuroendocrine origin of the tumour. Of the 27 chromogranin A-negative NET, 18 were histopathologically classified according to Capella *et al*. (1995): 50% of them (9/18) were high-grade malignant while of the other nine NET, eight were low-grade malignant and only one of them benign. Chromogranin A positivity (5-YSR: 81%, 10-YSR: 65%) was associated with a significantly better clinical outcome ($P=0.0105$; Fig. 6A) than negativity (5-YSR: 67.5%; 10-YSR: not determined). The Ki67-labelling index for the proliferating fraction of tumour cells was available for evaluation in 64.9% (259/399). The staining results were subgrouped: Ki67 index $<5\%$ (133/259; 51.4% of all cases), 5–10% (67/259; 25.9%) and $>10\%$ (59/259; 22.8%). The higher the Ki67 indeces were associated with, a significantly poorer clinical outcome ($P<0.0001$; Fig. 6B). This was also true in NET of unknown primary tumour localization (Ki67$<5\%$: 5-YSR 100%; Ki67$>10\%$ 5-YSR 46%, $P=0.03$).

The results of survival analysis according to the histopathological classification by Capella *et al*. (1995) are given in Table 4. Cumulative survival rates could only be compared between low- and high-grade malignant NET only because no statistically relevant events (i.e. deaths) occurred in the groups of benign NET and NET with benign or uncertain behaviour. Survival was significantly better for low- than high-grade malignant NET ($P=0.0001$; Fig. 7). Estimated 2- and 5-YSR for low- and high-grade malignant NET as well as for the two most abundant subgroups, ileal and pancreatic NET, are given in Table 4. According to the WHO classification (Solcia *et al*. 2000), 22.1% (30/136) were classified as well-differentiated endocrine tumours, 61.8% (84/136) as well-differentiated endocrine carcinomas and 15.4% (22/136) as poorly differentiated endocrine carcinomas.

Outcome of surgical intervention

Surgical procedures with curative intent were performed in 61.7% (198/321). R0 resection was achieved in 72% (139/193). After median interval of

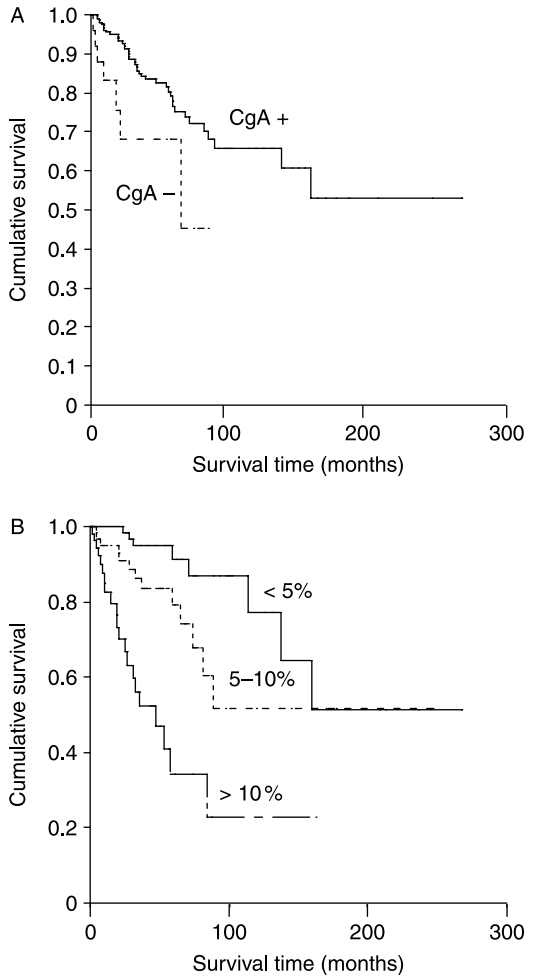

Figure 6 Cumulative survival (in months) according to histological criteria of (A) positivity (solid line) versus negativity (broken line) of immunohistochemical chromogranin A (CgA) staining ($P=0.0105$) or (B) Ki67-labelling index $<5\%$ (solid line), 5–10% (broken line)and $>10\%$ (partially dashed line; for *P* values, see Table 5).

20.6 months (range 1.2–186.7 months) 52 of the R0-resected patients experienced tumour recurrence, but only eight of these (8/52; 15.4%) were local recurrences while the others were newly diagnosed distant metastases. No significant differences were found in both number of recurrences and time to recurrence depending on foregut- versus midgut NET. Independent from its results, performance of any oncological surgical procedure was associated with a significant survival benefit ($P=0.0105$). Detailed survival analysis after resection revealed a better outcome in patients with R0 resection (5-YSR: 100%) without recurrence as compared with R0 resection with tumour recurrence (5-YSR: 92%; $P=0.0557$), R1/R2 resection (5-YSR: 74%;

Table 4 Influence of clinicopathological classification according to Capella *et al.* (1995) on 2- and 5-year survival rates (YSR)

Tumour characteristics	Frequency (numbers (% of cases))	2-YSR	5-YSR
All NET	252		
Benign	29/252 (11.5%)	100%	100%
Benign or uncertain behaviour	7/252 (2.8%)	100%	100%
Low-grade malignant	188/252 (74.6%)	97%	85%
High-grade malignant	28/252 (11.1%)	48%	ND
Ileal NET	75/252 (29.8%)		
Benign	0/75 (0%)	–	–
Benign or uncertain behaviour	1/75 (1.3%)	–	–
Low-grade malignant	73/75 (97.4%)	98%	90%
High-grade malignant	1/75 (1.3%)	–	–
Pancreatic NET	70/252 (27.8%)		
Benign	0/70 (0%)	–	–
Benign or uncertain behaviour	2/70 (2.9%)	–	–
Low-grade malignant	61/70 (87.1%)	97%	82%
High-grade malignant	7/70 (10%)	47%	ND

ND, not determinable due to death; –, to few cases to be determined.

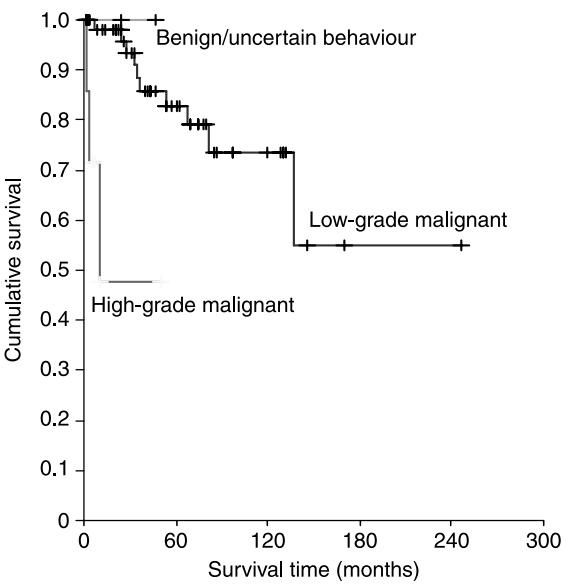

Figure 7 Estimated cumulative survival (in months; +, censored cases) according to clinicopathological classification by Capella *et al.* (1995).

$P=0.0042$), surgical tumour debulking without curative intent (5-YSR: 74%; $P=0.0007$) or without any surgical procedure (5-YSR: 54%; $P=0.0001$).

Independent prognostic factors of GEP-NET

Multivariate analysis of risk factors for NET-related death was performed after adjustment for age and gender using a Cox regression model as shown in Table 5. A primary tumour of >2.5 cm and presence of clinical symptoms were associated with an increased hazard ratio of death due to NET. Any performed oncological surgical procedure was associated with a decreased risk of death. In the subgroup for which a Ki67 index was available an increasing Ki67 index was associated with an increased risk of death due to NET. While presence of metastasis at initial diagnosis did not reach statistical significance on multivariate analysis for NET-related death ($P=0.0538$), it did reach statistical significance as a risk factor for first tumour progression after initial diagnosis by multivariate analysis (hazard ratio 4.120, 95% confidence interval: 2.074–8.187, $P<0.0001$).

Discussion

NET comprise a heterogeneous group of neoplasms with a rather long clinical course in the majority of the cases in spite of characteristics of malignancy (Kulke & Mayer 1999, Modlin *et al*. 2003, 2005, Klöppel *et al*. 2004). Specific hormone-related symptoms often make treatment mandatory even in the absence of tumour progression (Kulke & Mayer 1999, Plöckinger *et al*. 2004, Modlin *et al*. 2005). However, a subgroup of rapidly progressing NET exists and these patients need to be identified early and submitted to appropriate treatment. Less rapidly progressing patients also need to be clearly identified to prevent unnecessary and even potentially harmful treatment and to optimize the use of available treatment options (Kulke & Mayer 1999, Öberg *et al*. 2004*a*,*b*,*c*, Plöckinger *et al*. 2004, Modlin *et al*. 2005, Ramage *et al*. 2005). The reliability of prognostic parameters varies considerably in the studies published so far (Weber *et al*. 1995, Janson *et al*. 1997, Kirshbom *et al*. 1998, Madeira *et al*. 1998, Johanson *et al*. 1999, Shebani *et al*. 1999, Yu *et al*. 1999, Levi *et al*. 2000, Onaitis *et al*. 2000, Hemminki & Li 2001, Quaedvlieg *et al*. 2001, Modlin *et al*. 2003, Soga 2003, 2005, Van Gompel *et al*. 2004, Pape *et al*. 2004, Panzuto *et al*. 2005), which can be attributed to a number of reasons including heterogeneous study populations, heterogeneous disease definitions, varying clinicopathological classifications and incomplete datasets.

In this centre-based analysis, we carefully studied a well-characterized cohort of patients with NET with respect to various parameters. However, some characteristics of our centre have to be considered. First, because most patients are referred to our gastroenterological–oncological centre, a selection bias towards advanced, metastasized and even unsuccessfully pretreated patients occurs. Second, patients with small NET, which can be cured by simple surgical or

Table 5 Independent prognostic factors for neuroendocrine tumours (NET)-related death with associated cumulative 5- and 10-year-survival rates (YSR; according to Kaplan–Meier analysis) by univariate (log-rank test) and multiple analysis (Cox regression analysis; HR, hazard ratio; CI, confidence interval) and associated

Statistical test	Log-rank test			Cox regression		
Prognostic factor	**5-YSR (%)**	**10-YSR (%)**	***P* value**	**HR**	**95% CI**	***P* value**
Primary tumour <2.5 cm	88	74				
versus			0.0006			
Primary tumour >2.5 cm	68	44		4.44	2.1–9.3	<0.001
Absence of symptoms at initial diagnosis	100	100				
versus			0.026			
Presence of symptoms at initial diagnosis	77	58		8.2	1.1–63.5	0.049
Ki67 index <5%	96	78				
versus			0.03			
Ki67 index 5–10%	80	53		3.99	1.3–12	0.010
versus			<0.0001			
Ki67 index >10%	35	24		24.8	8–27.6	<0.001
Surgery performed	82	66		0.21	0.11–0.39	<0.001
versus			0.0105			
No surgery performed	56	47				

endoscopic intervention (e.g. appendiceal or rectal NET), rarely become referred to our centre because they can relatively easily be cured by the diagnosing centre and are therefore under-represented in our cohort. Third, because of a focus on pancreatic surgery at our hospital, a referral bias towards pancreatic NET seems possible. On the other hand, this cohort provides a large and well-characterized group of NET with a relatively long observation period, reliable clinical information, survival data and detailed information on the histopathological diagnosis. To our knowledge, this is one of the largest clinical cohorts of NET from a single centre analysed for clinical outcome parameters and prognostic factors.

Tumour characteristics

The general characteristics of the studied cohort are comparable with other series with an almost even gender distribution and an only slightly younger age of 54 years at initial diagnosis as compared with reported age at onset between 55 (Soga 2005) and up to 62 (Janson *et al*. 1997, Modlin *et al*. 2003) years. Therefore, the general composition of the cohort is unlikely to show unexpected age- or gender-related biases. Gender distribution is almost identical to data given in population-based studies (Modlin *et al*. 2003, Soga 2005) for the most frequent NET (ileum, pancreas and appendix). Only that of gastric NET was slightly different from the figures given by Modlin *et al*. (2003) but comparable with the centre-based data given by Granberg *et al*. (1998).

Primary tumour localizations within the cohort as given in Table 1 show an unusually high number of pancreatic NET which is higher than 79 pancreatic carcinoids reported by Modlin *et al*. (2003) in their extensive analysis of 13 715 carcinoid tumours. In the Niigata Registry for gut-pancreatic endocrinomas, Soga reported only 156 pancreatic carcinoids out of 12 540 cases of endocrine carcinomas stored in this registry (Soga 2005). Therefore, both the absolute number and the percentage of pancreatic NET is surprisingly high in our cohort compared with reports in the literature, particularly in comparison with population-based studies (Levi *et al*. 2000, Hemminki & Li 2001, Quaedvlieg *et al*. 2001, Modlin *et al*. 2003). When compared with other centre-based studies, only two groups reported pancreatic primary tumours at a relatively high frequency (11.1%; Kirshbom *et al*. 1998, Onaitis *et al*. 2000) and 43% (Panzuto *et al*. 2005). Table 6 gives a comprehensive comparison of primary tumour localizations in the available studies. Although the population-based data report a relatively low number of pancreatic NET, more recent centre-based studies (Onaitis *et al*. 2000, Pape *et al*. 2004, Panzuto *et al*. 2005) report a higher incidence of pancreatic NET. One likely reason is an under-estimation of the incidence of pancreatic NET when 'carcinoid tumours' thus classified by previous NET classifications were analysed. The WHO classification of 1980, in particular, distinguished carcinoid tumours from islet cell tumours (Klöppel *et al*. 1996, 2004). This led to the separation of carcinoid tumours, including pancreatic carcinoid tumours from endocrine pancreatic or islet cell tumours that were likely not included in the studied cohorts, and thus under-represented in the analyses. This is particularly true for population-based studies (Levi *et al*. 2000, Quaedvlieg *et al*. 2001, Modlin *et al*. 2003), but in centre-based studies such a selection bias may also have played a role (Janson *et al*. 1997, Kirshbom *et al*. 1998, Van Gompel *et al*. 2004). However, this concept has changed because of the tumour biological relations between these entities (Klöppel *et al*. 1996, 2004, Wiedenmann *et al*. 1998). This has led to a common classification as neuroendocrine tumours or carcinomas in the clinicopathological classification by Capella *et al*. (1995) and the latest WHO classification of 2000 (Solcia *et al*. 2000) and non-carcinoid pancreatic neuroendocrine tumours were consequently included in our study.

In contrast to pancreatic NET, the number of appendiceal NET is relatively low in comparison with the literature (see Table 6). As already mentioned, this is very likely due to an under-representation by a centre bias (Janson *et al*. 1997, Levi *et al*. 2000, Quaedvlieg *et al*. 2001, Modlin *et al*. 2003). Interestingly, the numbers reported by Panzuto *et al*. (2005) are very similar and probably represent a similar setting.

Finally, in our cohort, we observed a relatively high number of patients with unknown primary tumour localization which, however, is in accordance with most of the population-based studies (see Table 1; Levi *et al*. 2000, Quaedvlieg *et al*. 2001, Modlin *et al*. 2003) and therefore less likely presents a referral bias. NET with unknown primary tumour localization may have been under-reported in many of the centre-based studies because of the focus of these studies on gastrointestinal NET (Johanson *et al*. 1999, Shebani *et al*. 1999, Onaitis *et al*. 2000, Van Gompel *et al*. 2004, Panzuto *et al*. 2005) or even more specific on gastric (Granberg *et al*. 1998), pancreatic (Soga 2005), duodenopancreatic (Madeira *et al*. 1998) NET or even duodenopancreatic NET with Zollinger–Ellison's syndrome (Weber *et al*. 1995, Yu *et al*. 1999). Because of

Table 6 Comparison of distribution of primary tumour localization (as per cent of total) between Charité-results and other population- and centre-based studies

Type of study	Centre based	Population based					Centre based				
Site of primary	Charité ($n=399$)	The Netherlands ($n=2391$) Quaedvlieg 2001	Sweden ($n=5184$) Hemminki 2001	USA ($n=13\,175$) Modlin 2003	Japan ($n=11\,842$) Soga 2003	Switzerland ($n=433$) Levi 2000	Madison, USA ($n=97$) Van Gompel 2004	Durham, USA ($n=434$) Kirshbom 1998 and Onaitis 2000	Boston, USA ($n=150$) Shebani 1999	Uppsala, Sweden ($n=301$) Janson 1997	Rome, Italy ($n=156$) Panzuto 2005
Foregut	**46.1**			**33.7**			**14.4**	**22.0**	**5.3**	**13.0**	**48.7**
Bronchial	5.8	21.8	9.0	25.4	20.0	18.2				7.6	
Stomach	9.5	4.3	4.8	4.2	11.1	4.4	9.3	7.1	5.3	4.3	1.9
Duodenum	4.5			2.7			1.0	3.8	(stomach/		3.8
Pancreas	24.6	2.8	35.0	0.6	1.4	26.8	2.1	11.1	duodenum)		43.0
Midgut	**37.1**		(duodenum–	**35.3**	20.3	(duodenum–	**37.1**	**41.5**	**69.4**	**85.0**	**41.0**
Jejunum	2.8		ileum)	1.8	(duodenum–	ileum)	16.5	3.1			26.9
Ileum)	26.1	14.6		14.8	ileum)		(jejunum– ileum)	35.3	38.7		(jejunum– ileum)
Appendix	5.5	27.4	22.6	12.7	9.4		17.5		30.7		5.1
Caecum	2.5			3.9			3.1	3.1			
asc. Colon	0.3		11.6	0.9							0.6
Hindgut	**4.5**	11.3	(colon)	**18**	17.2	40.2	**15.5**	**6.7**	**25.3**	**2.0**	**8.3**
Rectum	4.5	(colorectum)	7.7	14.2	(colorectum)	(colorectum)	7.2	6.7	12.0		8.3
Extraintestinal	**1.8**	**5.1**	**9.3**	**1.2**	**12.6**	**10.4**	**32.9**				
Unknown primary	**10.5**	**12.1**		**11.8**	**3.6** (others)	(others)	(others)	**29.8**		**7.6**	

this, little is known about the clinical outcome of this subgroup of NET. Only in one study, a detailed analysis has been performed and our data differ with respect to longer 5- and 10-YSR of 74 and 55% as compared with 35 and 22% respectively in the study by Kirshbom *et al*. (1998). Unfortunately, no information is given on the grade of differentiation or other histological parameters in this study (Kirshbom *et al*. 1998). Therefore, it is unclear why survival was less favourable in their study. The data from our study suggest that histopathological differentiation grade plays an important role in this subgroup as is the case in all other NET.

Primary tumour localization has not uniformly been described as a prognostic factor. While some authors found pancreatic primary tumour localization to be a risk factor for decreased survival (Johanson *et al*. 1999, Onaitis *et al*. 2000, Modlin *et al*. 2003, Panzuto *et al*. 2005), others have not observed such a relationship (Van Gompel *et al*. 2004). However, although the difference in 5- and 10-year survival rates between pancreatic and ileal NET as the most abundant entities in our cohort did not reach statistical significance, a tendency towards a shorter survival in pancreatic NET could clearly be observed. This observation was even strengthened by the fact that the time to progression after initial diagnosis of NET disease was statistically significantly shorter in pancreatic than in ileal NET. In comparison with the available literature on pancreatic NET, the observed clinical outcome is nevertheless relatively satisfactory even though a high number of metastatic cases were included. The reported rate of metastatic disease of pancreatic NET lies between 60% (Panzuto *et al*. 2005), 66.7% (Soga 2005), 72% (Modlin *et al*. 2003), 77% (Madeira *et al*. 1998), 80% (Johanson *et al*. 1999) and 94.3% (Onaitis *et al*. 2000); therefore, the observed rates of metastasis in pancreatic NET (77% at initial diagnosis and 84% during the whole course) are comparable with other population- and centre-based studies as are survival rates (see Table 1). The 5-YSR in other studies were 28.9% (Soga 2005), 37.5% (Modlin *et al*. 2003), 62% (Panzuto *et al*. 2005) and 70% (Madeira *et al*. 1998). Population-based data show a worse prognosis for pancreatic NET than centre-based data. This can, in part, be explained by a selection bias towards selection of unfavourable subgroups of pancreatic NET and exclusion of favourable pancreatic NET such as functional NET that manifest earlier because of hormone hypersecretion syndromes (e.g. insulinomas). Also improved care of the complex pancreatic NET disease by centre-based management may contribute to these differences. For ileal NET, 5-YSR were within the range given in the literature (see Table 1). The 5-YSR in other studies were 55% (Van Gompel *et al*. 2004), 60.5% (Janson *et al*. 1997), 63% (Levi *et al*. 2000), 68% (Johanson *et al*. 1999) and 89.9% (Panzuto *et al*. 2005). Thus, we confirm that ileal NET generally have a better long-term outcome than pancreatic NET, although factors such as grade of malignancy and extent of metastatic disease have to be considered. In gastric NET, the numbers for each subgroup were rather small; however, our results are largely in accordance with the literature (Granberg *et al*. 1998, Onaitis *et al*. 2000, Modlin *et al*. 2003, Delle Fave *et al*. 2005).

In our cohort, presence of metastasis at initial diagnosis is an independent prognostic factor for tumour progression by both uni- and multivariate analysis and significantly influences survival by univariate analysis (Fig. 5) as has already been suggested by both population- (Levi *et al*. 2000, Quaedvlieg *et al*. 2001, Modlin *et al*. 2003, Soga 2005) and centre-based studies (Granberg *et al*. 1998, Janson *et al*. 1997, Kirshbom *et al*. 1998, Johanson *et al*. 1999, Van Gompel *et al*. 2004).

Confirmation of prognostic factors by multivariate analysis, however, comes from only a few centre-based trials with limited number of patients (Madeira *et al*. 1998, Yu *et al*. 1999, Panzuto *et al*. 2005). To our best knowledge, this is one of the largest published cohorts performing multivariate analysis of prognostic factors of gastroenteropancreatic NET identified by univariate analysis (see Table 5). Besides identification of metastasis as a significant prognostic factor for tumour progression, we could also confirm by multivariate analysis that a tumour size of more than 2.5 cm is an independent prognostic factor for NET-related death of the GEP confirming results by few other studies (Madeira *et al*. 1998, Shebani *et al*. 1999, Yu *et al*. 1999, Panzuto *et al*. 2005; see Fig. 3).

Clinical appearance

Clinical symptoms play an important role in diagnosis and management of NET (Capella *et al*. 1995, Janson *et al*. 1997, Kirshbom *et al*. 1998, Kulke & Mayer 1999, Onaitis *et al*. 2000, Hemminki & Li 2001, Soga 2003, 2005, Van Gompel *et al*. 2004, Öberg *et al*. 2004*c*, Pape *et al*. 2004, Plöckinger *et al*. 2004, Modlin *et al*. 2005, Panzuto *et al*. 2005). This study highlights a few aspects of clinical symptomatology with respect to making the diagnosis of NET disease, specificity of symptoms and functionality by hormone hypersecretion in particular, and the impact of symptoms on the prognosis of NET.

Observed clinical symptoms are largely in accordance with data from the literature and it is important to be aware of the fact that non-specific clinical symptoms are the most prevalent symptoms in NET patients (Janson *et al*. 1997, Kirshbom *et al*. 1998, Shebani *et al*. 1999, Onaitis *et al*. 2000, Soga 2003, 2005, Van Gompel *et al*. 2004, Modlin *et al*. 2005, Panzuto *et al*. 2005). Among these, abdominal pain is the most abundant symptom in 24–60% of patients. Not surprisingly, the rate of symptoms is the lowest (24%) in a study of early small gastrointestinal NET (Soga 2003) and is among the highest in our (55%) and other centre-based studies (57 and 60%; Kirshbom *et al*. 1998, Van Gompel *et al*. 2004), which include a large number of advanced NET, i.e. NET with loco-regional or distant metastasis (86.2% in our cohort). Even more importantly, we confirm previous studies showing a relatively low prevalence of specific symptoms related to hormone hypersecretion, i.e. functionality, at initial diagnosis and during the whole course of the disease, indicating that approximately two-thirds of NET are non-functional. This has been observed similarly by other centre-based studies (Kirshbom *et al*. 1998, Panzuto *et al*. 2005), although numbers for presence of functionality vary significantly between 14% (Van Gompel *et al*. 2004) and 71% (Janson *et al*. 1997) and strongly depend on the cohort characteristics.

Absence of clinical symptoms, whether non-specific or specific is also an independent prognostic factor associated with favourable survival in our cohort by uni- and multivariate analysis. This likely reflects a higher number of early-stage NET in this group, which are diagnosed either incidentally or because of transient non-specific complaints. It also underlines the importance of early diagnosis in NET.

Histopathology and classification

Histopathology including immunohistochemistry is the cornerstone of the diagnosis of NET and is required for a proper oncological diagnosis prior to management decisions (Lloyd & Wilson 1983, Wiedenmann *et al*. 1986, Capella *et al*. 1995, Klöppel *et al*. 1996, 2004, Pelosi *et al*. 1996, Kulke & Mayer 1999, Rindi *et al*. 1999, 2006*a*,*b*, Solcia *et al*. 2000, Van Eeden *et al*. 2002, Hochwald *et al*. 2002, Soga 2003, 2005, Öberg *et al*. 2004*c*, Plöckinger *et al*. 2004, Bajetta *et al*. 2005, Modlin *et al*. 2005) and, thus, was the crucial inclusion criteria for all patients in this study.

Immunohistochemistry for vesicular marker proteins is the most important study for verification of the neuroendocrine nature of the tumour (Lloyd & Wilson 1983, Wiedenmann *et al*. 1986, Capella *et al*. 1995, Solcia *et al*. 2000, Rindi *et al*. 2006*b*), and immunohistochemical findings were available for all patients. However, in external pathology departments, only one of the two marker protein was frequently considered sufficient for the diagnosis of a neuroendocrine neoplasm and therefore staining for both marker molecules (chromogranin A and synaptophysin) was not available for all cases. Interestingly, all NET stained for synaptophysin were positive by immunohistochemistry while this was true for only 90.8% of NET with chromogranin A staining. Suspected neuroendocrine nature of the tumour could be confirmed by synaptophysin staining in chromogranin A-negative cases. These results are in concordance with the literature (Wiedenmann *et al*. 1986, Klöppel *et al*. 1996, 2004, Van Eeden *et al*. 2002, Soga 2005). It is important that 50% of the chromogranin A-negative cases were classified as high-grade malignant by the classification by Capella *et al*. (1995) indicating a poor cellular differentiation. This translated into chromogranin A negativity as an independent risk factor for NET-related death by multivariate analysis and shorter survival rates (Fig. 6A). To our knowledge, this has not been shown by statistical analysis previously.

Besides making the definite diagnosis of the neuroendocrine nature of NET, it is also crucial to characterize their proliferative potential because this has been shown to be of prognostic relevance by several studies (Rindi *et al*. 1999, Van Eeden *et al*. 2002, Hochwald *et al*. 2002, Jorda *et al*. 2003, Klöppel *et al*. 2004). Most of the clinical series published, however, have not studied histopathological parameters that are indicative of tumour cell differentiation, in detail. In our cohort, we analysed the Ki67 index in 259 patients, almost two-thirds of the study population. As is shown in Fig. 6B and Table 5, Ki67 index is a strong and independent prognostic factor which confirms results from previous studies (Pelosi *et al*. 1996, Hochwald *et al*. 2002, Jorda *et al*. 2003).

Another aspect revealed by our analysis is a good correlation between clinical outcome and clinicopathological categories of two classification systems, namely the one proposed by Capella *et al*. (1995) and the WHO classification of 2000 (Solcia *et al*. 2000). This provides important proof from a large clinical series that these clinicopathological classification systems are of prognostic relevance in routine clinical settings. This has been similarly judged by others although clinical data are still very limited (Van Eeden *et al*. 2002, Hochwald *et al*. 2002, Bajetta *et al*. 2005). However, clinical series frequently have described the extent of metastatic disease as a prognostically relevant feature (Kirshbom *et al*.

1998, Onaitis *et al*. 2000, Van Gompel *et al*. 2004, Pape *et al*. 2004, Panzuto *et al*. 2005, Soga 2005). However, a widely applied systematic classification system was not available until recently. In 2006, a proposal for a TNM classification including a Ki67 index or mitotic index-based grading system was proposed (Rindi *et al*. 2006*b*), and prognostic relevance was recently confirmed in a first study (Pape *et al*. 2008). A detailed histopathological description of NET and a detailed registration of the extent of tumour load will therefore likely be one of the most important prognostic systems in the near future (Rindi *et al*. 2006*a*,*b*).

Outcome of interventions

Clinical outcome in patients with NET is strongly influenced by therapeutic interventions. However, frequently multiple interventions occur because the course of the disease is long and severity of clinical symptoms, recurrent or progressive tumour lesions require repeated interventions (Öberg *et al*. 2004*a*,*b*,*c*, Plöckinger *et al*. 2004, Modlin *et al*. 2005, Ramage *et al*. 2005). Retrospective analysis is therefore a method with limitations for validation of therapeutic strategies. Surgical intervention after initial diagnosis can be evaluated best because it usually occurs at the beginning of the disease and usually no other interfering specific therapeutic strategies are applied at the time of surgery (except for anti-symptomatic treatment). The positive results of surgical intervention with curative intent underline the importance of evaluation of the possibility of surgery in every patient. Survival is significantly better for operated patients and any possibility to actively improve prognosis for NET patients should be discussed probably in an interdisciplinary setting (Plöckinger *et al*. 2004). Although initial surgical success rate with curative R0 resection was 72%, 35% of these patients experienced tumour recurrence after a median interval of ~20 months, therefore decreasing the long-term success of surgery to below 50%. Follow-up time in this retrospective analysis is, however, rather short for validation of long-term success and prospectively designed analyses are required for a thorough analysis. However, other surgical studies have reported similar results (Lo *et al*. 1996, Janson *et al*. 1997, Johanson *et al*. 1999, Soreide *et al*. 2000, Hellman *et al*. 2002, Schindl *et al*. 2002).

Conclusions

In conclusion, we demonstrate in a retrospective analysis of patients with mainly advanced gastroenteropancreatic NET that size of the primary tumour, expression of the immunohistochemical marker chromogranin A and the Ki67 index are independent prognostic factors for long-term survival of patients with NET. Presence of metastasis is an independent risk factor for tumour progression after initial diagnosis. Taken together, these factors represent key features in the combined clinicopathological classification systems by Capella *et al*. (1995) and the WHO (Solcia *et al*. 2000). We also found absence of clinical symptoms at initial diagnosis, a 'routine parameter' in the clinical evaluation of NET patients, who were helpful in predicting a favourable clinical outcome. Neither functional syndromes that are not as frequent as thought previously, nor primary tumour localization are independent predictors of long-term outcome. Therapeutic intervention and particularly early curative surgery do have an impact on long-term survival of NET patients and should therefore be actively planned in all patients. Finally, this analysis has identified important prognostic factors that are relatively easily obtainable and therefore reveals new information for the development of prognostically relevant strategies to diagnosis, risk stratification and therapeutic management in patients with NET in the near future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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