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LETTER TO THE EDITOR

A short report of 50 patients with gastroenteropancreatic mixed neuroendocrine–non-neuroendocrine neoplasms (MiNEN)

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Introduction

Gastroenteropancreatic (GEP) mixed neuroendocrine–non-neuroendocrine neoplasms (MiNEN), are rare tumors [1,2] comprising a neuroendocrine and a non-neuroendocrine component, both accounting for at least 30% of the neoplasm [3,4]. The non-neuroendocrine component is most often an adenocarcinoma but may be composed of all types of malignant epithelial tumors found in the digestive system [4]. The neuroendocrine component is classified according to the WHO 2019 classification of neuroendocrine neoplasms and is subdivided into three grades based on the Ki67 proliferation index and differentiation [1]: well differentiated neuroendocrine tumors, i.e., NET G1 (Ki67 $<$ 3%), NET G2 (Ki67 3-20%), and NET G3 (Ki67 $>$ 20%) and poorly differentiated neuroendocrine carcinomas, i.e., NEC (Ki67 $>$ 20%). Most MiNENs contain poorly differentiated NEC.

It has been proposed that the prognosis of GEP MiNEN is driven mostly by the neuroendocrine component and its Ki67 proliferation index [5]. Another tool for choosing the right therapeutic strategy is to assess the grade of malignancy of each cellular component [3,4]. Based on this approach, it has been proposed to divide GEP MiNENs into three categories [3,4]. The high-grade malignant MiNEN is composed of a poorly differentiated NEC with a non-neuroendocrine component [4,6]. The intermediate-grade MiNEN combines a well-differentiated NET G1, G2, or G3 with a non-neuroendocrine carcinoma [4,6]. The low-grade MiNEN combines a well-differentiated NET with an adenoma [4,6].

Due to poor awareness and limitations in diagnostic methods, the incidence of MiNENs may be underreported. There are currently no published data on the incidence of MiNENs and published international treatment guidelines do not exist. The aim of this single center retrospective analysis is to describe the clinical and pathological findings and to analyze treatment outcomes of a cohort of GEP MiNEN patients.

Material and methods

Inclusion criteria

Patients with GEP MiNEN referred from 1 January 2011 to 31 December 2017 to the Neuroendocrine Tumor Center of Excellence, Copenhagen University Hospital, Rigshospitalet, were included. MiNEN-patients with predominantly abdominal metastases from an unknown primary (CUP) were also included.

Ethics

The study was approved by the Danish Data Protection Agency at Rigshospitalet (ref. no. 2007-58-0015) and by the Danish Patient Safety Authority (31-1521-453). Due to the retrospective design, informed consent approval from the Ethical Committee was not required.

Data collection

Data were collected from the prospective NET database at Rigshospitalet and the Danish National Pathology Database. Data included gender, age at diagnosis, date of diagnosis, performance status (PS) at diagnosis, location of primary tumor, metastases, and TNM-classification at diagnosis. Surgical and oncological treatment data were retrieved from the date of diagnosis until date of death or end of follow-up, dated on the 21 January 2020. Progression-free survival (PFS), recurrence-free survival (RFS), and overall survival (OS) were recorded. The surgical data included the type and anatomical location of the surgical intervention and its radicality and oncological data. The pathological data included Ki67 proliferation index, pTNM (pathological TNM-staging), cTNM (clinical TNM-staging) in patients not eligible for resection, large vs. small cell morphology, tumor differentiation (poorly vs. well-differentiated morphology), determination of largest

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tumor component, and the results of immunohistochemical staining for synaptophysin, chromogranin A, and Ki67 in the NEN component [7].

Study endpoints and statistical analysis

The primary endpoints were OS, RFS, and PFS. OS was defined as the time from date of diagnosis until death of any cause. RFS was defined as the time from date of radical surgery until recurrence or death of any cause. PFS defined as from start of treatment until progression or death. The Kaplan–Meier plots and log-rank tests were used for survival analyses. Multivariable Cox regression analyses were performed as stepwise regression with backward elimination. PS, stage of disease, and surgical intervention was analyzed using the multivariable Cox regression. Age and gender were excluded from the multivariable Cox regression as they were not significant predictors in the univariable models. All statistical analyses were performed using IBM SPSS v. 25 (Armonk, NY, USA). p Values of $<.05$ were considered significant.

Results

Patient population

Fifty consecutive patients were included with a median follow-up time of 33 (1–104) months. Patient characteristics are presented in Table 1. Thirty-six patients (72%) had local disease at diagnosis and 14 patients (28%) had disseminated disease. The most frequent metastatic manifestations at diagnosis and during follow-up were liver metastases (18 patients) and peritoneal carcinomatosis (15 patients). All tumors were positive for synaptophysin (>30% reactive cells), and 27/50 (54%) were positive for CgA. No patients had hereditary syndromes.

Twenty-one patients were evaluated with fluorodeoxyglucose-positron emission topography (FDG-PET). Pathological FDG uptake was detected in 16 of 21 (76%) patients at diagnosis or during follow-up, with lesions visible on CT or MRI. Furthermore, eight patients were evaluated with either ^{64}Copper DOTATATE PET or ^{68}GalliumDOTATOC PET. Pathological uptake was observed in one patient (12.5%) during follow-up and no pathological uptake was observed at the time of diagnosis.

Survival

At the end of follow up, 31 of 50 (62%) patients had died. The median OS for all patients was 31 months (1–104 months) (Figure 1(A)). Twenty-eight of 31 (90%) of the deceased patients suffered from a MiNEN related death. The 30-day mortality rate was 0%.

In the 32 patients who were radically operated (R0), the median RFS was 32 months (3–84 months) (Figure 1(B)) and median OS was 47 months (4–104 months). The median follow-up time of the radically operated patients was 39 months (4–104 months). Seventeen of the 32 (53%) patients had relapse during follow-up. The median PFS of the 32 patients with disseminated disease at diagnosis, recurrence during follow-up, or untreatable illness at diagnosis, was 3 months

Table 1. Patient characteristics, stage distribution, component distribution and pathological findings, surgical and oncological data, metastases at diagnosis and the development of metastases during follow-up.

Patient characteristics	
Female/male	22/28
Age, median (range)	68 years (27–84)
Upper GI (esophagus, cardia, stomach), *n*	20 (F/M 3/17)
Lower GI (small intestine, appendix, colorectal), *n*	26 (F/M 15/11)
Pancreas, *n*	2 (F)
Unknown primary, *n*	2 (F)
Stage distribution at diagnosis	
Local and locally advanced stage (M0)/advanced stage (M1)	36 (72%)/14 (28%)
Distribution of tumor component and pathological findings	
NEN > non-NEN	15 (30%)
NEN = non-NEN	4 (8%)
NEN<non-NEN	17 (34%)
Assessment of component distribution not available	14 (28%)
Median Ki67 index	90% (4–100%)
Upper GI, median Ki67 index	100% (30–100%)
Lower GI, median Ki67 index	80% (4–100%)
Pancreas, Ki67 index	60% and 100%
Unknown primary, Ki67 index	Both are 100%
Synaptophysin, positive samples, *n* (%)	50 (100%)
Chromogranin A, positive samples, *n* (%)	27 (54%)
Adenocarcinoma with NEN, *n* (%)	48 (96%)
Squamous cell carcinoma with NEN, *n* (%)	2 (4%)
Surgery	
Upper GI, R0 surgical resection, *n*	12 (60%)
Lower GI, R0 surgical resection, *n*	19 (73%)
Pancreas, R0 surgical resection, *n*	1 (50%)
Chemotherapy	
Perioperative NEC-regimen (platin-etoposide)	7
Perioperative upper/lower GI-regimen (5-fluorouracil based)	15
Palliative chemotherapy only	14

NEN: neuroendocrine neoplasm; GI: gastrointestinal; NEC: neuroendocrine carcinoma.
Data are presented numerically and when appropriate as percentage.

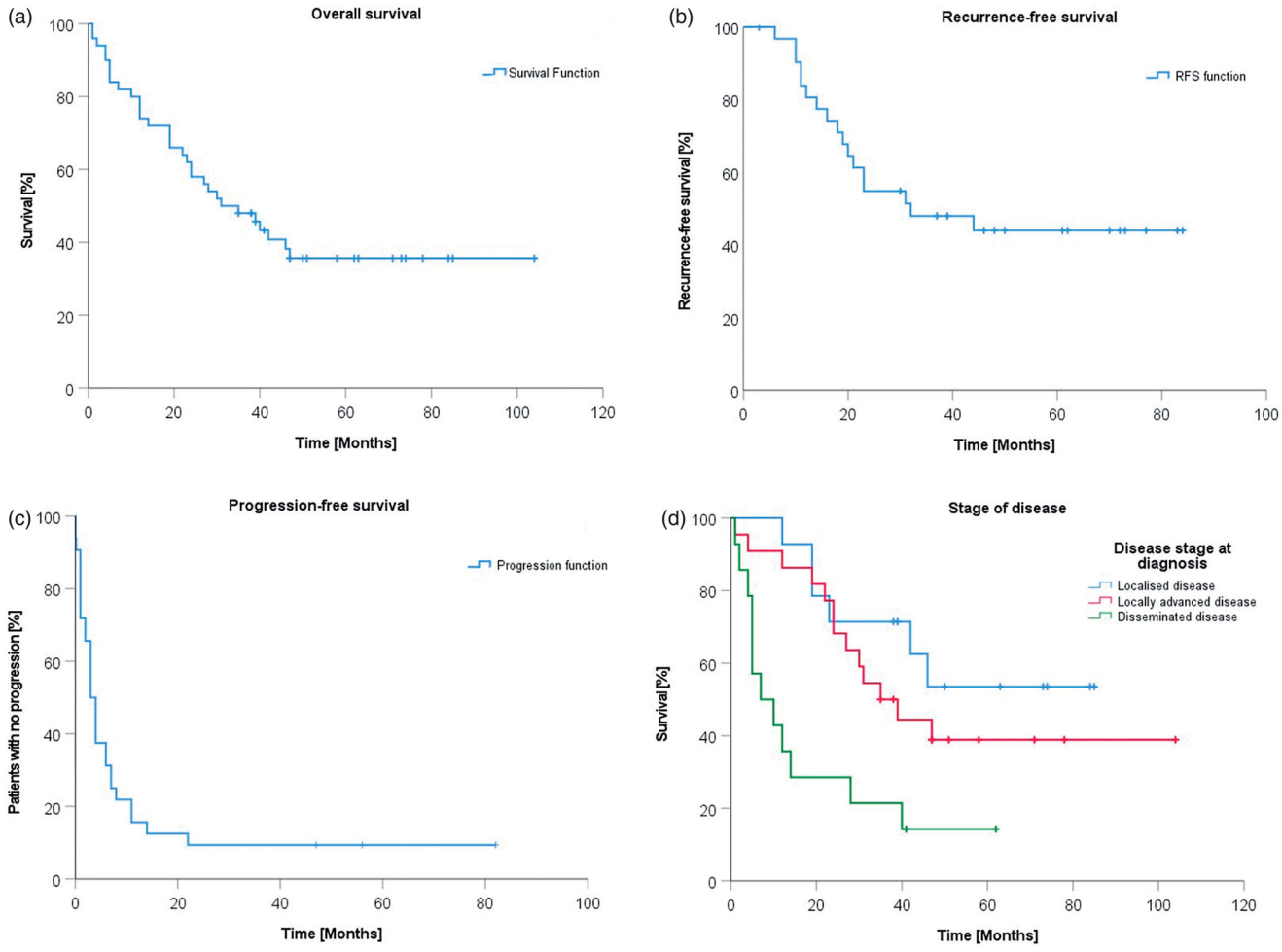

Figure 1. Overall survival of all patients included in the study (a, $N = 50$), recurrence-free survival of patients with radically resected tumors (b, $N = 32$), progression-free survival of all patients with disseminated disease at diagnosis or during follow-up (c, $N = 32$), and overall survival based on the stage of disease at diagnosis (d, $N = 50$).

(<1–82 months) (Figure 1(C)). In the 11 unresectable patients with disseminated disease at diagnosis, median OS was 5 months (1–41 months).

General prognostic factors in the entire cohort

Stage of disease, surgical resection, and PS at diagnosis were statistically significant prognostic factors. The stage of disease was grouped in three categories: localized disease, local disease with regional lymph node metastases, and disseminated disease. Survival was longer in patients with localized disease or local disease with regional lymph node metastases compared to patients with disseminated disease ($p = .002$). Patients with only localized disease had a similar median OS compared to patients with localized disease and regional lymph node metastases (44 months vs. 35 months, $p = .36$). Patients with radically resected tumors had longer median OS than patients who did not undergo surgery (47 months vs. 5 months, $p = .002$). After adjustment for surgical treatment, stage of disease and PS at diagnosis, we found that a poorer PS at diagnosis (HR: 2.1 (1.3–3.2, 95% CI) $p = .001$) and no surgical intervention (HR: 3.4 (1.5–7.8, 95% CI) $p = .004$) were statistically significant predictors of mortality. An interaction test was performed including the variables PS at diagnosis and surgical treatment. The results showed a p value of .18 and thus indicating no interaction between these two variables, although it should be noted that the statistical precision is limited due to the small sample size. When comparing gender in an independent samples t-test, a statistically significant difference of survival between the two groups could not be observed ($p = .064$), also Cohen's D effect size was 0.127 indicating that gender has a small effect on OS. There was no statistically significant difference in median OS between patients with a primary lower GEP-MiNEN vs. patients with an upper GEP-MiNEN (42 months vs. 24 months, $p = .25$). Chromogranin A level at diagnosis had no statistically significant impact on median OS ($n = 45$, $p = .87$). The dominant tumor component in the GEP MiNEN lesion had no impact on survival with no statistically significant difference in median OS between patients having tumors with majority of NEN tumor cells vs. majority of non-NEN tumor cells ($p = .35$). Two-thirds (21 of 32) of the R0-resected patients had perioperative chemotherapy. The drug regimens adhered to the localization of the primary and to the dominant tumor component. The sample sizes are however too small to elaborate further on the efficacy.

Discussion

One of our main findings was that almost half of the operated patients did not show evidence of recurrence during follow-up, and a substantial number of the patients were long term survivors. Most recurrences occurred within the first two post-operative years. Our results highlight the importance of considering patients for surgery. As expected, generally the PFS in patients with disseminated disease was short. The median OS for the entire cohort of GEP MiNEN patients was 31 months; 46 months for patients with local disease at diagnosis and 7 months for patients with disseminated disease. The prognosis of MiNEN patients has been evaluated in few retrospective studies. The results on OS differ between these studies: in three of the largest retrospective reports concerning GEP MiNEN ($n = 160$, $n = 96$, and $n = 69$), the median OS across the entire cohorts was 13 months, 45 months, and 16 months, respectively [5,8,9]. Data on RFS were reported in two of the studies and in both studies the RFS was lower than reported in our population, 14 months and 11 months, respectively, vs. 32 months in this study [8,9]. These results highlight the heterogenicity of this disease entity.

It has been proposed that the prognosis of MiNEN is primarily determined by the neuroendocrine component, especially if it consists of NEC, which is most frequently found in MiNEN [1,4–6,9]. Accordingly, the prognosis of patients with MiNEN has been suggested to be similar to the prognosis of patients with NEC [4–6]. In a study of 2546 patients with GEP-NEC from the Surveillance, Epidemiology and End Results Program database (SEER), registered from 1973 to 2012, the median OS of patients with regional disease was 16 months. Patients with disseminated disease at diagnosis had a median OS of 5 months [10]. Another study of 305 GEP NEC patients diagnosed between 2000 and 2009 showed that the median OS for patients with advanced disease receiving palliative chemotherapy was 11 months [11]. Based on our results, the prognosis of GEP MiNEN mirrors that of GEP NEC. A recent study showed that MiNEN has a worse prognosis compared with patients with pure NEC in the small intestine and in the appendix but no statistically significant difference in OS between the two in other parts of the GI-tract [12].

In a study of patients with high-grade malignant MiNEN, a NEN component of more than 50% of the total tumor size was a poor prognostic factor and could predict the presence of tumor embolisms and liver metastases [13]. We could not confirm this result. When comparing lesions with a synaptophysin reactivity above 50% (NEN-dominant lesions) with lesions showing synaptophysin reactivity below 50% (non-NEN dominant lesions), we found no difference in median OS. Thus, we speculate that prognosis may not depend on the amount of each cellular component, but primarily on other factors such as a poorly differentiated cell morphology of the NEN component and a Ki67 proliferation index $\geq$55%, which is in line with findings by others [4,5].

In our study, most MiNEN originated from the colon-rectum ($N = 26$) and the esophagus and gastroesophageal junction ($N = 20$) which is in agreement with the distribution found by others [5,9]. We did not find any statistically significant difference in OS between gastroesophageal GEP MiNEN vs. colorectal MiNEN.

Strengths and limitations

Strengths of the study include that no patients were lost to follow up, the cohort is well characterized with a long median follow-up period, detailed clinical data concerning all patients and a central pathology review performed by a pathologist specialized in GEP NEN tumors. The study represents a single-center study covering GEP MiNEN patients from approximately 50% of the Danish population. However, due to the limited size of diagnostic biopsies, not all MiNENs are expected to be captured. The retrospective nature of the study and the cohort size of only 50 patients represent indisputable limitations, even though the patients were included successively and thus did not undergo selection bias.

Conclusions

GEP-MiNEN is a rare and heterogeneous disease entity. Regardless of the tumor composition, patients should be evaluated for surgical treatment as it is associated with the best prognosis. Patients with disseminated GEP MiNENs have a very poor prognosis.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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