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ORIGINAL SCIENTIFIC REPORT

Natural History of MEN1 GEP-NET: Single-Center Experience After a Long Follow-Up

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Abstract

Background The multiple endocrine neoplasia type 1 syndrome (MEN1) natural history is poorly evaluated, and few single-institution experiences about hereditary gastroenteropancreatic neuroendocrine tumors (GEP-NET) are reported. Our purpose is to analyze the role of GEP-NET in MEN1-related death, as well as the behavior of these lesions during follow-up.

Methods The study population consists of 77 patients diagnosed with MEN1 GEP-NET, regularly followed up since 1990. Extensive clinical data were prospectively recorded. Statistical analysis was performed both on the whole population of 77 patients and on two subgroups including patients who, during the long lasting study period, underwent GEP-NET surgery (50 pts) and who did not (27 pts), respectively.

Results Twenty-five males (32.5%) and 52 females (67.5%) were enrolled. Sixty-four patients had MEN1 family history (83.1%), and genetic mutation was detected in 67 cases (87%). The mean age at GEP-NET diagnosis was 41.4 years (SD = 13.6); 16 patients (20.8%) had GEP-NET diagnosed before age 30 and 12 cases (15.6%) before 1996. The mean interval time between MEN1 diagnosis and GEP-NET detection was 5.7 years (range −11/37; SD = 8.1 years). Overall, the mean follow-up time from MEN1 diagnosis was 15.8 years (SD = 9.7 years) and from GEP-NET diagnosis was 9.6 years (SD = 6.9 years). Gastrinoma was the most frequent functioning GEP-NET and pancreatoduodenectomy the most adopted surgery. GEP-NET progression affected 12 patients within the non-surgical group, while 18 subjects developed progression after surgery.

Conclusions Our single-center data provide information on epidemiologic, clinical and pathological features of GEP-NET in MEN1 making possible to clarify their natural history.

Introduction

Multiple endocrine neoplasia type 1 syndrome (MEN1) is an autosomal dominant genetic disorder. *MEN1* gene is located on chromosome 11q13, and it was identified in 1997 [1–5]. Gastroenteropancreatic neuroendocrine tumors (GEP-NET) arise early in patients affected by MEN1. Two forms of GEP-NET are described in MEN1: non-functioning tumors (NF), characterized by the absence of production and/or release of neuroendocrine polypeptides, and functioning tumors, producing active hormones such as gastrin, insulin, vasoactive intestinal polypeptide (VIP), glucagon or somatostatin, giving rise to a typical clinical syndrome [6, 7].

The GEP-NET malignancy is variable, depending not only on the size but also on histological type; it seems to be low for lesions smaller than 2 cm in diameter and for insulinomas (12–20%), but it is relevant for NF tumors and gastrinomas (about 70%) [6–9].

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Unlike sporadic GEP-NET, many small endocrine tumors (condition referred to as microadenomatosis) are generally present in the pancreas of patients with MEN1. The pancreatic microadenomas are often associated with macrotumor/s (diameter ≥ 1 cm), some of which may be functional. The GEP-NET penetrance in MEN1 is high, reaching 80–90% at 60 years of age, similarly to primary hyperparathyroidism (PHPT) [6–11].

Different imaging examinations are conducted in order to assess GEP-NET progression. However, endoscopic ultrasound (EUS) is an operator-dependent second-level procedure and trans-abdominal ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) have low sensitivity in detecting lesions less than 1 cm in diameter, thus leaving most of the small GEP-NET potentially unrecognized [12–24].

Furthermore, in patients affected by MEN1, even in presence of functioning GEP-NET, the frequent association of other non-functioning pancreatic lesions prevents the localization of the tumor responsible for hormone production through purely morphological techniques.

Although scientific progress occurred in both MEN1 GEP-NET diagnosis and treatment, the natural history of the disease remains poorly evaluated [10, 25–28].

The purpose of this study is to analyze the role of GEP-NET in MEN1-related death, as well as the behavior of these lesions during follow-up.

Materials and methods

The study population consists of 77 patients affected by MEN1 diagnosed with GEP-NET, belonging to various families, regularly followed up at the Department of Surgery and Translational Medicine, University Hospital Careggi, Florence, Italy, in the period 1990–2014. Clinical data were prospectively recorded in an electronic database after signature of informed consent by all the study participants and after obtaining ethical approval by the local Experts Committee of Careggi Hospital. Family trees have been established for all index cases and used to identify the affected family members. According to international guidelines [3, 29], the following criteria were considered for GEP-NET diagnosis in MEN1:

- Patients with a detected *MEN1* mutation affected by GEP-NET, either associated or not with other neuroendocrine tumors (parathyroid, pituitary, adrenal, thymic, bronchial, gastric lesions).
- Patients with clinical/radiologic evidence of GEP-NET belonging to a known MEN1 family with at least one first-degree-relative affected by a neuroendocrine tumor.
- Patients with negative genetic test and without MEN1 family history but affected by GEP-NET plus PHPT and/or pituitary adenoma.

The diagnostic criteria to define the presence of GEP-NET in MEN1 and thus to establish the inclusion into the present study were clinical (medical history and physical examination, blood tests and stimulation test with secretin) and/or radiologic [US, CT, MRI, somatostatin receptor scintigraphy (SSRS), EUS] and/or pathological.

Each patient underwent an extensive examination regarding signs and symptoms due to GEP-NET. A medical history was meticulously collected.

Time of MEN1 onset was defined as the time of the first clinical manifestation of MEN1 (renal colic, pituitary disease, hormonal syndrome or radiologic evidence of GEP-NET, etc.) or, in case of asymptomatic patients, the time when the disease was detected by biochemical screening or genetic test.

Clinical visit, blood examinations for baseline enteropeptidase and hormones [neuron-specific enolase (NSE); chromogranin A (CgA); pancreatic polypeptide (PP); somatostatin (SS); vasoactive intestinal polypeptide (VIP); glucagon; insulin and c-peptide, gastrin and basal acid production (BAO)] were performed yearly at our Center. US was performed every 12 months, while abdominal CT or MRI and EUS were performed every 12–24 months, SSRS as needed. The secretin stimulation test was performed every 12–18 months by intravenous injection of 75 IU synthetic human secretin (Secrelux®, Goldman, Neuss, Germany). Blood samples were taken at baseline, 2.5, 5, 7.5, 10, 15 and 30 min after secretin administration.

This scheduled follow-up (clinical, biochemical and radiologic) was adopted to evaluate or diagnose pituitary tumors, primary hyperparathyroidism (PHPT), GEP-NET and/or adrenal gland disease. When suspected, the presence of liver metastasis was confirmed by histology [high prevalence in patients with MEN1 of focal nodular hyperplasia (FNH)]; thoracic CT and/or SSRS and/or MRI and histology were adopted for thymic carcinoid evaluation; the presence of bronchial carcinoids was assessed by fibrobronchoscopy and/or thoracic X-ray and/or CT and/or SSRS and/or MRI and histological confirmation; gastric carcinoid was diagnosed by upper digestive endoscopy with biopsy. MEN1-associated skin lesions (collagenomas, angiofibromas, lipomas), as well as any other neoplastic lesion, were recorded in the electronic dataset.

Surgical intervention for GEP-NET was indicated in case of either clinical syndrome or imaging showing lesions over 1.5–2 cm in diameter or significant increase in volume (a doubling of tumor size exceeding 1 cm over a 12-month interval) (Fig. 1). The preoperative finding of

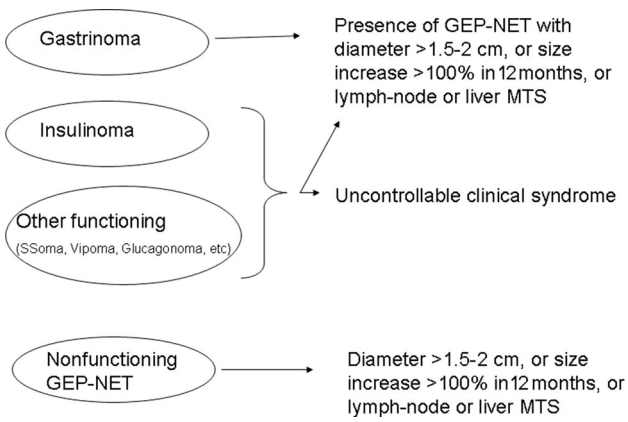

Fig. 1 Indications to surgery according to the different preoperative clinical characteristics of GEP-NET

liver lesion/s was not considered a contraindication. At surgery, intraoperative US evaluating both pancreas and liver was performed. In patients with gastrinoma, a particular attention was deserved to the duodenum: Kocher maneuver, palpation and transillumination were performed.

All surgical operations aimed to remove every functioning GEP-NET and all the eventually present concomitant macroscopic lesion/s found within the pancreas or in regional lymph nodes and liver.

The number and size of each measurable GEP-NET described at pathological examination were recorded.

The occurrence of new GEP-NET or a significant increase in volume (>25%) or the evidence of secondary lymph node involvement or liver metastasis was defined as disease progression, regardless of the presence or absence of a typical endocrine syndrome. In the surgical cohort, GEP-NET progression was evaluated from the time of primary surgery. In presence of disease progression to liver, we indicated surgery whenever possible, while medical therapy (with a strict radiologic follow-up) was performed in presence of major liver involvement in patients with scarce performance status.

Adrenalectomy was indicated for lesions either larger than 4 cm in diameter or having atypical or suspicious radiologic features and 1–4 cm in diameter, or showing rapid growth.

Initial clinical status as well as follow-up data including clinical features, secretin test, imaging findings, surgical procedures, medical therapies prescribed for GEP-NET was recorded for each patient.

Data analysis

Statistical analysis was performed on all collected data: Regarding time-related variables, the time of the event was defined as the interval between the birth (time zero) and the occurrence of the studied event (MEN1 diagnosis, evidence of GEP-NET, GEP-NET progression, death). The following factors were analyzed in relationship to GEP-NET progression and mortality: age at MEN1 diagnosis, age at GEP-NET diagnosis and at surgery; age over 30 at MEN1 diagnosis; age over 30 at GEP-NET diagnosis; MEN1 diagnosis before 1996; GEP-NET diagnosis before 1996; family history; gender; presence of MEN1 mutation; type of mutation; MEN1 diagnosis/GEP-NET diagnosis interval

Table 1 Data of the study cohort (77 patients)

No. of patients	77 (25M, 52F)
Positive family history	83%
Mean age at MEN1 diagnosis	35.3 years (SD = 12.8) under 30 = 28 (36.4%) diagnosed before 1996 = 30 (38.9%)
Mean age at GEP-NET diagnosis	41.4 years (SD = 13.6) under 30 = 16 (20.8%) diagnosed before 1996 = 12 (15.6%)
Mean MEN1 GEP-NET diagnostic interval time	5.7 years (range −11/36; SD = 8.1 years)
Diagnostic tool	Imaging: 90.9%; biochemical assay: 9.1%
Associated syndromic features	
PHPT	70 (90.9%)
Pituitary adenoma	47 (61%)
Adrenal tumor	9 (11.7%)
Bronchial carcinoid	6 (7.8%)
Mean follow-up from MEN1 diagnosis	15.8 years (SD = 9.7)
Mean follow-up from GEP-NET diagnosis	9.6 years (SD = 6.9)
Mortality	4 patients (5.2%)
Causes of death	
GEP-NET progression	2 cases (2.6%)
Thymic carcinoid	1 case (1.3%)
Pulmonary embolism	1 case (1.3%)

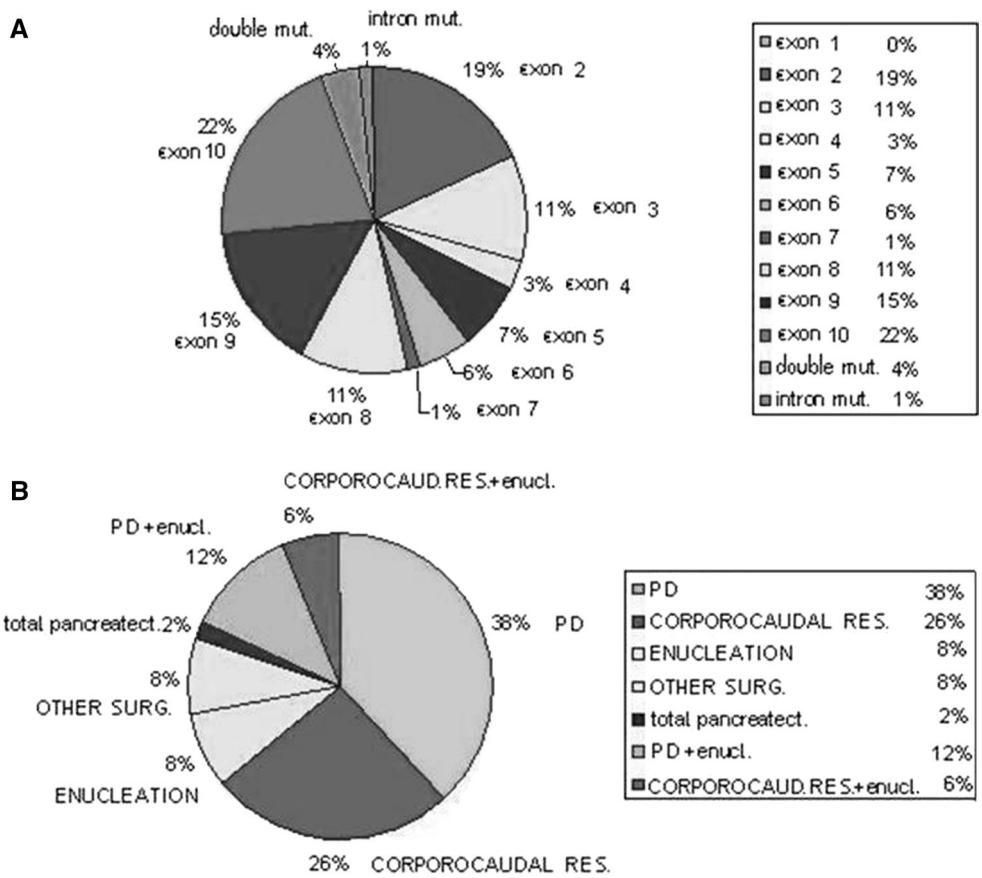

Fig. 2 **a** Pie chart showing the percent distribution of *MEN1* mutations in the 77-patient cohort and **b** pie chart showing the primary surgery performed in 50 cases

time; GEP-NET diagnosis/surgery interval time; age over 50 at the last follow-up; positive preoperative secretin test; initial syndrome symptoms; type of GEP-NET (gastrinoma, insulinoma, other); surgery for GEP-NET; presence of lymph node metastasis at surgery; presence of liver metastasis at surgery; surgery before 1996; type of surgery [pancreatoduodenectomy (PD), corporocaudal pancreatic resection; GEP-NET enucleation; total pancreatectomy; PD plus enucleation; corporocaudal resection plus enucleation; liver surgery for metastasis or FNH]; surgical complications; maximal diameter of lesions $<$ or $>$ 1.5 cm; adrenal surgery; carcinoid exeresis; re-operation for recurrent disease; positive secretin test at surgical follow-up; concordance between radiologic imaging and secretin test; disease progression; interval time between GEP-NET diagnosis and progression. This statistical analysis was conducted both on the whole population of 77 patients overall and on the subgroups of operated and not-operated cases.

PHPT was not an object of the statistical analysis because almost all the patients were affected.

All the clinical variables were considered time-dependent covariates. The time cutoff 1996 for the variable MEN1 or GEP-NET diagnosis was arbitrarily chosen according to the change in diagnostic and therapeutic strategies for patients affected by MEN1 syndrome, due to the positional cloning of *MEN1* gene.

For all the analysis performed, $p < 0.05$ was considered significant.

Results

Our cohort included 77 patients with MEN1, 25 males (32.5%) and 52 females (67.5%). Clinical data are reported in Table 1. A genetic mutation was detected in 67 cases (87%) (Fig. 2a).

The mean age at MEN1 diagnosis was 35.3 years (SD = 12.8 years); the mean age at GEP-NET diagnosis was 41.4 years (SD = 13.6 years). In seven patients (9.1%), GEP-NET diagnosis relied on biochemical basis (secretin test positive for one or more entero-hormones), while in 70 patients (90.9%) imaging examinations confirmed the diagnosis. However, 20 of these patients (28.6%) during the study follow-up were not eligible for surgery based on both clinical and radiologic criteria.

Table 2 Clinical features of surgical group by type of GEP-NET primary operation

Type of operation	No. pts	Clinical syndrome	Mean age at surgery	Postoperative complications	Postoperative progression
PD ± enucl.	25	19 ZES 3 hypoglycemia 1 WDHA[a] 2 symptomless	45.39 years (range 28.02–63.22)	9 (36%) 5 surgically treated 4 medically treated	6 (24%)
Corporocaudal resection ± enucl.	16	1 ZES 7 hypoglycemia 8 symptomless	37.19 years (range 19.34–62.76)	9 (56%) 3 surgically treated 6 medically treated	4 (25%)
Enucleation only	4	1 ZES 2 hypoglycemia	28.87 years (range 17.44–44.21)	1 (25%) surgically treated	3 (75%)
Total pancreatectomy	1	1 ZES	45.03 years	1 (100%) medically treated	0 (0%)
Other	4	2 ZES 2 symptomless	51.10 years (range 41.02–69.41)	0 (0%)	2 (50%)

[a] *WDHA* watery diarrhea, hypokalemia, achlorhydria

Overall, the mean follow-up time from MEN1 diagnosis was 15.8 years (SD = 9.7 years), and the mean time from GEP-NET diagnosis was 9.6 years (SD = 6.9 years).

Surgical cohort

Data regarding the group of patients who required GEP-NET surgery ($n = 50$, 71.4%) are described in Tables 2 and 3. The mean age at surgery was 40.6 years (SD = 12.4). A PD was performed in 19 patients (38%), corporocaudal resection in 13 cases (26%), enucleation of one or more pancreatic lesions in 4 patients (8%), PD plus enucleation in 6 cases (12%), corporocaudal resection plus enucleation of pancreatic head tumors in 3 patients (6%), total pancreatectomy only in one female patient (2%) affected by multiple (n. 23) lesions found within all the pancreatic gland, most of which >1.5 cm in diameter. The same patient was also affected by gastrinomas metastasis on the VII liver segment, radically treated. Four patients (8%) required a different surgical treatment: one underwent ileal resection for NF GEP-NET; the other three, presenting duodenal gastrinomas, underwent gastroduodenal resection, partial duodenectomy and endoscopic exeresis, respectively (Fig. 2b). At primary surgery, liver involvement (minor/segmental) was found in 7 patients (14%): Synchronous resection was possible in all cases; however, histological examination of the specimen showed FNH in 4 of these patients (57.1%). Nine patients (18%) underwent adrenal surgery.

The evaluation of the preoperative clinical syndrome combined with pathological and immunohistochemical analysis on the specimen, allowed the identification of 28 patients (56%) affected with gastrinoma, 15 (30%) with insulinoma, 2 (4%) with vipoma, 1 (2%) with glucagonoma. The other 4 patients (8%) were affected by non-functioning GEP-NET only.

Analyzing these 50 operated patients, a mean of 17.12 NF lesions per patient (range 0–62) were described at the macroscopic plus microscopic examination of the surgical specimen.

Postoperative complications and diabetes mellitus incidences are reported in Table 3.

Within the surgical subgroup, a GEP-NET oncologic progression occurred in 16 patients (32%) after a mean postsurgical follow-up of 6.6 years (range 1–17 years); in all cases, it was assessed by imaging studies (CT, MRI, SSRS, EUS, US), while secretin test was positive in only 6 cases (37.5%). Two patients (12.5%) died due to unoperable liver progression. Only three patients (6%) required surgery for GEP-NET progression: The first case was a young woman who, four years after PD, developed para-aortic lymph node metastasis from gastrinoma; the second patient, 2 years after PD performed to treat Zollinger–Ellison syndrome (ZES), developed hepatobiliary tree gastrinoma with ZES recurrence, treated with hepatic resection of segments V-VIII; the third patient, instead, after a first enucleation of a pancreatic head lesion in 1995, underwent multiple operations: In 1996 further enucleation associated with liver surgery for lesions which were shown to be FNH; in 2004 duodenal mucosectomy to treat ZES; in 2008 ampullectomy, then in 2009 PD associated with radiofrequency (RF) ablation of a single small liver metastasis. For the other 11 patients (68.7%), the presence of residual pancreatic lesions did not represent, after 3.8 years (range 2–7 years) mean follow-up from the detection of GEP-NET progression, a surgical indication, being the symptoms manageable with medical therapy (somatostatin analogues) or the lesion smaller than 1.5–2 cm in diameter.

Table 3 Surgical cohort data (50 patients)

No. of patients	50 (71.4%)
Mean age at MEN1 diagnosis	35.1 years (SD = 12)
Mean age at GEP-NET diagnosis	39.3 years (SD = 12.3)
Mean MEN1 GEP-NET diagnostic interval time	1.3 years (SD = 2.9 years)
Positive secretin test	31 cases (62%)
Symptoms related to functioning GEP-NET syndrome	38 cases (76%)
Mean follow-up from MEN1 diagnosis	16.6 years (SD = 9.3)
Mean follow-up from GEP-NET diagnosis	12.0 years (SD = 7.3)
Mean age at surgery	40.6 (SD = 12.4)
Type of surgery	
PD	19 (38%)
Corporocaudal resection	13 (26%)
Enucleation	4 (8%)
PD + enucleation	6 (12%)
Corporocaudal resection + enucleation	3 (6%)
Total pancreatectomy	1 (2%)
Further abdominal surgery	4 (8%) [1 ileal resection; 3 gastroduodenal surgery]
Liver resection	7 (14%) [FNH in 4 cases, 57.1%]
Adrenal surgery	9 (18%)
Postoperative complications	20 pts (40%)
Conservatively treated	11 pts (55%)
Surgically treated	9 pts (45%) (bleeding 1; pancreatitis/fistula 5; anastomotic leakage 2; internal hernia 1)
Postoperative diabetes mellitus	6 pts (12%)
Pathological examination	
Gastrinomas	28 pts (56%)
Insulinomas	15 pts (30%)
Vipomas	2 pts (4%)
Glucagonoma	1 pt (2%)
NF only	4 patients (8%)
Mean no. of NF per patient	17.12 (range 0–62)
Maximum GEP-NET diameter	Range 0.1–5 cm; >1.5 cm in 16 cases (32%)
Liver metastases	3 pts (6%)
Lymph node metastases	10 pts (20%)
GEP-NET progression	16 pts (32%)
Increase in number or syndrome development	10 pts (62.5%)
Increase in volume	2 pts (12.5%)
Evidence of liver or lymph-nodal metastasis	4 pts (25%)
Mean progression time	6.6 years (range 1–17)

Concerning the 77 patients analyzed in our database, 4 of them died a mean of 5 and 5.7 years after diagnosis of MEN1 and GEP-NET, respectively (range 0.1–11 years). All of those patients had undergone surgery for GEP-NET: the first patient, operated on of gastroduodenal resection for ZES in 1989, died in 2000 aged 52 for progression of thymic carcinoid; the second, 72-year-old, died in 2001, 9 years after PD, due to liver metastases from GEP-NET; the third, operated at the age of 61 for liver metastases from NF GEP-NET, died in 2003, 3 years after surgery; the fourth patient, aged 53, died in 2003 because of pulmonary embolism during the short-term postoperative course after PD associated with liver resection for synchronous liver metastases.

Non-surgical cohort

A separate analysis was also performed on the 27 patients (28.6%) who did not meet surgical criteria or had not yet undergone surgery for GEP-NET at the end of this study

Table 4 Non-surgical group data (27 patients)

No. of non-surgical patients	27 (28.6%) [8M (29.6%), 19F (70.4%)]
Mean age at MEN1 diagnosis	35.6 yrs (SD = 14.4); over 30 = 17 pts (63%); diagnosed before 1996: 9 (33.3%)
Mean age at GEP-NET diagnosis	45.4 yrs (SD = 15.3); over 30 = 23 pts (85.1%); diagnosed before 1996: no cases
Mean MEN1 GEP-NET diagnostic interval time	9 years
Syndromic symptoms	1 case (3.7%)
Mean follow-up from MEN1 diagnosis	15 years (SD = 10.5)
Mean follow-up from GEP-NET diagnosis	5.2 years (SD = 2.9)
Positive familial history	23 (85.2%)
Positive secretin test	21 (77.8%)
Type of positive hormone	
Gastrin	12 pts
Glucagon	5 pts
VIP	1 pt
NSE, PP, CgA	3 pts
GEP-NET progression	12 pts (44.4%)
Increase in number or syndrome development	10 pts (83.3%)
Increase in volume	2 pts (16.7%)
Evidence of liver or lymph node metastasis	0 pts (0%)
Mean progression time	3.3 years (range 1–13)

(Table 4). They were 19 females (70.4%) and 8 males (29.6%) with a mean age at diagnosis of MEN1 of 35.6 years (SD = 14.4), mean age at GEP-NET diagnosis of 45.4 years (SD = 15.3) and a mean age at last follow-up of 50.6 years (SD = 15). All the patients of this subgroup were alive at the end of the study. Family history of MEN1 was present in 23 cases (85.2%); *MEN1* mutation was identified in 22 patients, respectively, in exon 2 (5 cases), 3 (2 cases), 4 (1 case), 5 (1 case), 6 (1 case), 8 (5 cases), 9 (4 cases), 10 (4 cases), and a double mutation was found in 2 patients. At diagnosis of GEP-NET, a clinical syndrome was present in only one of them (3.7%) who showed ZES, but a secretin test was positive in 21 patients (77.8%); two patients (7.4%) did not performed the test and had radiologic diagnosis of GEP-NET.

According to the data related to the secretin test, a positive test for gastrinoma was present in 12 cases (with a simultaneous increase in the values of glucagon and CgA in 3 cases, PP in another patient, PP and CgA in another case).

In the non-surgical subgroup of 27 patients, GEP-NET progression occurred in 12 cases (44.4%), average 3.33 years after GEP-NET diagnosis (range 1–13 years). In three of these cases, the size of GEP-NET or the occurrence of an uncontrollable syndrome indicated surgical treatment, but two patients refused surgery (starting somatostatin analogues therapy) and the other one had not yet undergone GEP-NET surgery at the end of the study period.

Statistical analysis

Adopting the log-rank method (Mantel-Cox) on all 77 patients (Table 5), variables significantly associated with GEP-NET progression (considering follow-up after MEN1 diagnosis) were: male gender, MEN1 mutation in exon 8, diagnosis of MEN1 before 1996, surgery for GEP-NET, primary enucleation, PD + enucleation, secretin test positive at follow-up; diagnosis of GEP-NET performed before 1996 did not reach statistical significance ($p = 0.056$). Figure 3a shows the Kaplan–Meier curve for progression free survival of all 77 patients, adopting as follow up the time from GEP-NET diagnosis.

Adopting the log-rank method (Mantel-Cox) on 50 patients operated for GEP-NET, variables significantly associated with GEP-NET progression (follow-up calculated after GEP-NET diagnosis) were: MEN1 mutation in exon 8 ($p = 0.011$), familiar MEN1 ($p = 0.022$), diagnosis of MEN1 performed before 1996 ($p = 0.001$), enucleation surgery ($p = 0.026$) or DCP + enucleation ($p = 0.043$) and secretin test positive at follow-up ($p = 0.028$); in cases diagnosed as gastrinoma, the likelihood of disease progression was higher than cases with other GEP-NET ($p = 0.084$). In the subgroup of 50 patients operated for GEP-NET, the Kaplan–Meier curve showing GEP-NET progression (follow-up from primary surgery) is shown in Fig. 3b.

Adopting the log-rank method (Mantel-Cox) on 27 patients not operated on for GEP-NET, variables

Table 5 Risk factors (and relative confidence intervals) for GEP-NET progression observed in the study group (77 pts) by using the log-rank method (Mantel-Cox)

Risk factor	Confidence interval	Hazard ratio	*p* value
Gender male	Male versus female	2.22	0.035
	14.2–25.2 versus 24.1–32.2		
Mutation in exon 8 of *MEN1* gene	Mutation in exon 8 versus other mutation	1.89	0.036
	14.9–26.1 versus 24.4–28.0		
Diagnosis of MEN1 before 1996	Diagnosis before 1996 versus after 1996	9.76	0.001
	13.4–18.2 versus 28.4–35.9		
Surgery for GEP-NET	Surgery for GEP-NET versus other treatment	1.52	0.021
	20.5–26.3 versus 25.3–29.3		
Type of operation (enucleation)	Enucleation versus other	1.49	0.026
	19.7–23.9 versus 24.1–28.3		
Type of operation (PD + enucleation)	PD + enucleation versus other	2.44	0.043
	19.2–24.3 versus 23.5–28.8		
Positive secretin test at follow-up	Positive secretin test versus negative secretin test	1.18	0.028
	18.2–22.8 versus 24.1–27.9		

significantly associated with progression (follow-up after MEN1 diagnosis) were male gender ($p = 0.012$) and MEN1 diagnosis before 1996 ($p = 0.001$). Mutation in exon 4 and double mutation of *MEN1* did not reach significance ($p = 0.056$ and 0.064, respectively). Figure 3c shows Kaplan–Meier curve for GEP-NET progression in this subgroup of 27 patients (follow-up from MEN1 diagnosis).

Globally, the log-rank method (Mantel-Cox) on all 77 patients found the following variables significantly associated with mortality after GEP-NET diagnosis: MEN1 mutation in exon 8 ($p = 0.001$), DCP as first treatment ($p = 0.017$), presence of liver metastases at the first operation ($p = 0.007$), need for further surgery for liver metastasis ($p = 0.001$), presence of postoperative complications ($p = 0.02$), age at last follow-up >50 years ($p = 0.036$). Male gender ($p = 0.09$), positive preoperative secretin test ($p = 0.088$), presence of gastrinoma ($p = 0.08$) and positive secretin test at follow-up ($p = 0.09$) did not reach statistical significance. The overall survival Kaplan–Meier curve from the date of diagnosis of GEP-NET in all 77 patients is shown in Fig. 4.

Discussion

The gene *MEN1* was cloned only 15 years ago, and the genetic test is still negative in 10% of MEN1 families [30]. This is also confirmed by our data, as the 12.99% of the cohort had a negative *MEN1* genetic test. In MEN1 syndrome, no clear genotype/phenotype correlation has been described yet, but our data show *MEN1* mutation in exon 8 associated with both GEP-NET progression and mortality. Interestingly, the studied population is a representative sample of Italian people affected by MEN1, since treated patients were referred to our center from various national hospitals. However, referring to epidemiological aspects (age at diagnosis, sex ratio and the prevalence of GEP-NET lesions) the reported cohort does not differ greatly from other international case studies, such as the 233 patients with MEN1 followed at the Mayo Clinic [27].

Since it is well known that GEP-NET is the leading cause of disease-related deaths in patients with MEN1, this study aims to assess the impact of various histological types of GEP-NET on the outcome. Our data showed an increased risk of progression and death in patients with gastrinoma (all four patients who died were suffering from that tumor). Potential bias in this evaluation is the fact that a mean of 17.1 NF GEP-NET lesions were found at the macro- and microscopic analysis of the surgical specimen of the operated patients, and in 16 cases, those NF tumors were >1.5 cm in diameter. Some studies in fact demonstrate that NF GEP-NET lesions seem to be as aggressive as gastrinomas or even more [25–27, 31–33]. However, such aspect, which could be explained by a late diagnosis due to the absence of clinical symptoms, needs to be carefully assessed by future prospective studies carried out in specialized centers, in which secretin stimulation test and specific radiologic examinations are routinely performed, and immunohistochemistry is adequately conducted for GEP-NET analysis. In our series, the 4 patients affected only by NF GEP-NET showed no statistically different outcome compared to the patients with functioning GEP-NET associated with NF GEP-NET.

Notably, we surgically treated patients with MEN1 avoiding conservative approaches but aiming to grant

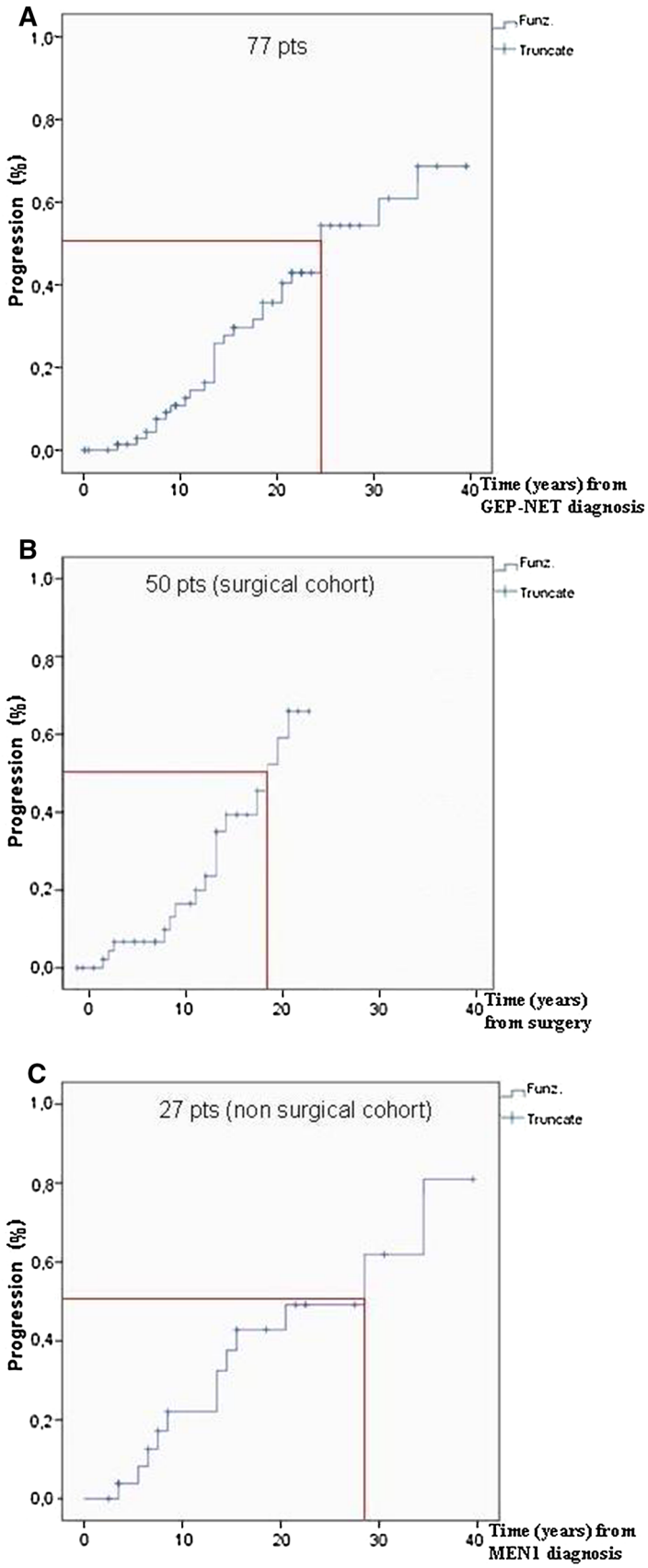

Fig. 3 **a** Kaplan–Meier curve showing oncologic progression in 77-patient cohort starting from time of diagnosis of GEP-NET. **b** Kaplan–Meier curve describing the oncologic progression in 50 operated patients with follow-up calculated from primary surgery. **c** Kaplan–Meier curve describing the oncologic progression in 27 non-operated patients with follow-up calculated from diagnosis of MEN1

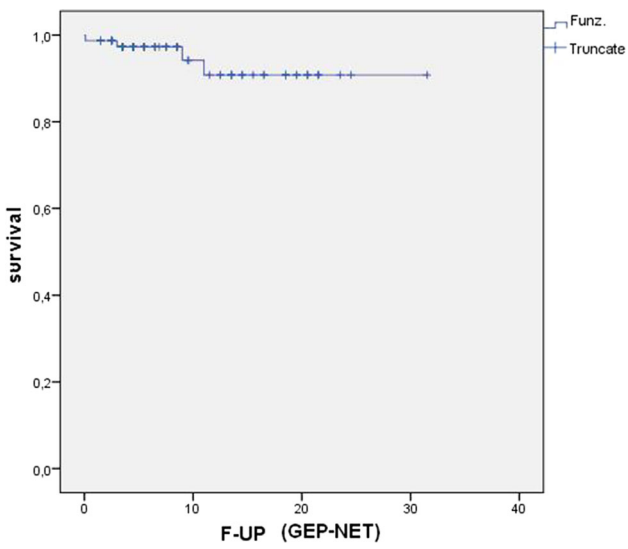

Fig. 4 Kaplan–Meier curve showing overall survival of 77 patients with follow-up calculated from the time of diagnosis of GEP-NET

oncologic radicality. In fact, among the 50 operated patients, disease progression of GEP-NET occurred in 32% of cases, after a mean postsurgical follow-up of 6.6 years. Insulinoma in MEN1 seems to be less aggressive than other types of GEP-NET, as none of the affected patients died during follow-up. Such behavior does not seem to be related to a size criterion (insulinomas are usually larger than 1.5 cm), but maybe to an earlier diagnosis and treatment because of the important clinical syndrome; another hypothesis suggests that a greater differentiation might be responsible for the indolent behavior [34]. However, the constant association with NF lesions observed in our cases highlights the need of further studies to assess the consequences of limiting the treatment of these cases to the simple enucleation of the insulinoma.

In our study, we also performed the statistical evaluation according to the size of the tumors (diameter $<$ or $>$ 1.5 cm, respectively) and the presence of lymph node and/or liver metastases. Only the presence of liver metastases and liver surgery had a significant impact on survival. Therefore, it is very important to differentiate between benign and malignant liver lesions, although differential diagnosis can be extremely difficult despite preoperative targeted imaging studies (MRI with liver-specific contrast). Therefore, an aggressive surgical attitude in patients with synchronous liver lesions could be of both diagnostic and prognostic value. Age at GEP-NET primary surgery was under 50 years in the vast majority of patients, thus supporting the need for an accurate evaluation and treatment of liver lesions.

Concerning the adrenal lesions and carcinoids, in our study they were not shown to increase significantly the risk of mortality. No deaths directly related to the adrenal gland involvement were recorded. These results, however, do not confirm what was already reported by Skogseid [35] and Langer [36], who observed a significant rate of adrenal hyperplasia evolution toward highly aggressive carcinomas in MEN1. One patient in our series died in 2000 at age 52 for recurrence of thymic carcinoid.

A statistical strength but also a possible limit of the present study is the long period of observation; in fact, it might introduce statistical bias due to epocal variations in MEN1 clinical management (diagnostic tools and therapeutic strategies); however, the mono-centric nature of the present study contributed to minimize the bias, as the patients were followed/treated by the same medical team. The statistical analysis took into account the period of diagnosis of MEN1 and GEP-NET, and the date of surgery, in order to accurately define the impact of each lesion on the risk of disease progression and death. Notably, a significant decrease in GEP-NET progression was observed in patients diagnosed with MEN1 after 1996, while age at follow-up over 50 was a significant risk factor for mortality, and a diagnosis of MEN1 before 1996 was more correlated with disease progression. This suggests that *time* has a key role in GEP-NET progression, even in operated patients and independently of the type of pancreatic surgery performed primarily. In fact, in our experience, both enucleation of GEP-NET and PD associated with enucleation were statistically related to disease progression. This aspect was adequately analyzed during the long postoperative follow-up in our series.

Goudet [28] found that the prognosis of MEN1 syndrome has improved regularly since 1980, with a slight decrease in MEN1-related mortality over time, respectively, 76.8 and 71.4% before and after 1990. This might be due to the drastic reduction in operative mortality and the virtual disappearance of deaths related to complications of ZES (perforation and/or bleeding). These mortality rates are, however, quite high compared to previous experiences reporting MEN1-related death rates between 28 and 46% [25–27]. Different length of median follow-up of these studies may explain this discrepancy. Ito [10] described NIH experience about 106 cases of ZES in MEN1 reporting 23% mortality at 24.5-year follow-up with no mortality linked to acid hypersecretion complications: Even if the surgical approach they adopted is not reported in details, in our cohort mortality rate is lower, allegedly due to the shorter mean follow-up time. In MEN1 GEP-NET (particularly in case of ZES), we prefer to perform aggressive surgery including regional lymphadenectomy, in order to remove all functioning tumors (and potentially functioning tissue) as well as every lesion larger than 1.5 cm.

In our series, though the low MEN1-related mortality rate (5.2%), we found, as expected, the presence of familiar history of MEN1 to have a protective effect on the risk of death, probably leading to an earlier diagnosis and surgery for GEP-NET. However, this factor had the opposite effect on the risk of progression, particularly in patients operated for GEP-NET. A possible explanation is the phenomenon of the anticipation linked to the genetic transmission.

Interestingly, regarding *time,* even in presence of a similar follow-up from MEN1 diagnosis (15 vs. 16.6 years in non-surgical and surgical cohorts, respectively), the behavior of GEP-NET seems heterogeneous, since some patients did not reach the surgical criteria, while in other cases, a disease progression occurred.

Even if secretin stimulation test is not considered a parameter to indicate surgery, we regularly performed it in the clinical management of patients: Our results show that a positive test is significantly related to the risk of progression, indicating its diagnostic sensibility and potential utility in the pre- and postoperative follow-up, especially to obtain a timely detection of GEP-NET hormone production, allowing both an early and potentially more accurate diagnosis and treatment.

Conclusion

The present paper, describing single-center data about MEN1 GEP-NET epidemiologic, clinical and pathological features, allows to clarify their natural history: GEP-NET are often multiple but surgically curable and tend to progression over time, even after surgery. Still unknown factors let them be clinically more aggressive in some patients (who require surgery) than in other. Scheduled surveillance allows a prompt surgical therapy able to minimize the rate of mortality.

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Author's contribution FG, TC and FG collected the data; GG, TC and FG analyzed the data; FG, TC, GB and FT wrote the manuscript; FT and MLB supervised the manuscript.

Compliance with ethical standards

Conflict of interest Authors certify that there is no actual or potential conflict of interest in relation to this article, and they state that there are no financial interests nor connections, direct or indirect, or other situations that might raise the question of bias in the work reported or the conclusions, implications, or opinions stated—including pertinent commercial or other sources of funding for the individual author(s) or for the associated department(s) or organization(s), personal relationships, or direct academic competition. The authors certify also that the manuscript, including related data and figures, has not been previously published and that it is not under consideration elsewhere.

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