Metastatic potential and survival of duodenal and pancreatic tumors in Multiple Endocrine Neoplasia Type 1. A GTE and AFCE cohort study. (Groupe d'étude des Tumeurs Endocrines and Association Francophone de Chirurgie Endocrinienne).

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Mini-abstract: This 603-patient series of MEN1 patients with duodeno-pancreatic tumors shows that Zollinger-Ellison-syndrome, large tumors over 2 cm, were associated independently to the risk of

metastases occurrence. Only tumors larger than 2 cm were significantly associated to an increase in the risk of death once distant metastases occur.

ABSTRACT

Objective: To assess the distant metastatic (d-met) potential of duodeno-pancreatic neuroendocrine tumors (DP-NETs) in patients with MEN1 according to their functional status and size using a multistate model to account for the overall natural history of MEN1 disease.

Summary background data: DP-NETs remain an issue of concern because of the multiplicity of lesions and symptoms related to endocrine secretion; unfortunately they may also turn into aggressive tumors associated with d-met, leading to shorter survival. Survival of large non-functional DP-NETs is known to be poor survival. But, the overall contribution of DP-NETs to d-met spread is poorly known.

Methods: The study population comprised all patients with a DP-NET diagnosed after 1990 and followed in the MEN1 cohort of the Groupe d'étude des Tumeurs Endocrines (GTE). A multi-state Markov piecewise constant intensities model was applied to separate the effects of prognostic factors on 1) d-met, and 2) d-emt-free death or 3) death after d-met occurrence.

Results: Among the 603 patients included, 39 had d-met at DP-NET diagnosis, 50 developed d-met during their follow-up and 69 died. The Markov model showed that Zollinger-Ellison-related tumors (regardless of tumor size), large tumors over 2 cm, and age over 40 years were independently associated with an increased risk of d-met occurrence. Men, patients over 40 years old and with tumor larger than 2 cm all had also an increased risk of death once d-met occurred.

Conclusions: DP-NETs reaching 2 cm in size, whatever associated secretion, should be removed to prevent d-met and to increase survival. Indication for surgery in case of gastrinoma remains under debate.

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) is an inherited disease that predisposes carriers to primary hyperparathyroidism (pHPT), duodeno-pancreatic tumors (DP-NETs), pituitary tumors, adrenal, thymic (th-NETs) and bronchial neuroendocrine tumors. The criteria for diagnosis were established first in Gubbio (1) and then regularly updated (2,3). MEN1 is related to mutations in the MEN1 gene, a gene of approximately 10-kb that encodes for menin and is located on chromosome 11q13 (4–6).

DP-NETs represent the second most frequent MEN1 associated lesion and are responsible for the majority of MEN1 cancer-related deaths (7–9). DP-NETs may be functional or non-functional. The two main associated secretions are gastrin, responsible for Zollinger-Ellison Syndrome (ZES), and insulin, caused by gastrinomas (ZES-DP-NETs) and insulinomas (ins-DP-NETs), respectively. Aggressive forms of ZES-DP-NETs exist and may shorten survival (10,11). By contrast, ins-DP-NETs do not seem to modify survival even though rare metastatic forms exist (8,12). Non-functioning pancreatic tumors (NF-DP-NETs) account for a large proportion of DP-NETs and their prevalence has increased as new imaging tools have become available. NF-DP-NETs seem to be more frequently associated with metastasis when their size at diagnosis is greater than 2 cm (13-16). DP-NETs of the glucagonoma, VIPoma and somatostatinoma group are large and frequently associated with metastatic spread but are extremely rare (17). Previous studies showed that gender and the JunD genotype may influence DP-NETs' survival (18,19). Nevertheless, the independent factors associated with the aggressiveness of DP-NETs with regard to metastatic spread and/or mortality remain not well known. However, to investigate factors associated with the risk of distant metastasis it is necessary to separate the effects of prognostic factors on metastasis on the one hand, and death attributable to other lesions or to acute symptoms related to endocrine secretion on the other hand. For this purpose, an adequate modelling to avoid competition between the two risks is required. Thus, the aim of this study was to assess the metastatic potential of DP-NETs and their impact on survival in patients with MEN1 according to their secretion and size, using a multistate model that accounts for the overall natural history of the MEN1-disease.

MATERIALS AND METHODS

Population

The study population comprised all patients with a DP-NET diagnosed after 1990 and followed in the MEN1 cohort of the Groupe d'étude des Tumeurs Endocrines (GTE) (**figure 1**).

This cohort has already been described (13,14,17-19). Briefly, the GTE network for MEN1, created in February 1991, includes the 22 reference clinical centers in France and Belgium as well as the four genetics departments in charge of MEN1 diagnosis. Data are gathered in a secure database at the Dijon Clinical Investigation Centre (INSERM CIC1432) and regularly controlled.

The MEN1 cohort was approved by the CCTIRS (Consultative Committee on Treatment of Information in Health Research, file number 12.364) and the CNIL (National Committee for Data Protection, authorization number DR 2013-348). Written consent is not required by the French law, but patients are informed about their right to withdraw their data from the cohort.

Definitions of MEN, duodeno pancreatic NETs and outcomes

In agreement with international guidelines (1,2), the following criteria were used to confirm MEN1 disease: 1) patients with a MEN1 mutation and having at least one MEN1-related lesion, 2) patients belonging to an already known MEN1 family in which at least one first-degree relative has been affected and who had at least one MEN1-related lesion, 3) patients without positive genetic testing and without a familial background, with at least two of the three major MEN1 lesions (pHPT, DP-NETs and pituitary tumors). Patients who presented only two major lesions were considered with caution since these associations may occur randomly in the general population. DP NETs could be diagnosed using various techniques: 1- MRI or CT-scan confirmed by specific imaging studies (scintigraphy), 2- histology obtained by: a- FNA during Endoscopy Ultrasound Sonography (EUS) or by resection using gastroduodenal endoscopy, b- by surgery, 3- Abnormal endocrine secretions (gastrin, insulin) since they were related to the presence of DP-NETs even though the precise location of the NETs could not always be found. (See ZES and insulinoma definitions in the supplemental material section).

In our study, the two outcomes of primary interest were the occurrence of distant neuro endocrine metastasis and death. For death, the exact date and cause were known. Metastasis was defined as distant metastasis and excluded isolated loco-regional invasion or node involvement. Date of the first diagnosis of metastasis corresponded to the date of the imaging or surgery at which the metastasis was identified.

Prognostic factors

Prognostic factors, evaluated at the time of DP-NET diagnosis, included gender, age (< 40 years vs ≥ 40 years), the presence of ZES or insulinoma, tumor size (≥ 2 cm vs < 2 cm) and the JunD mutation. The size of DP-NETs was measured according to the closest medical imaging within the three months before or after the diagnosis, given the slow evolution of MEN1-related tumors. In cases of secretion with no visible tumor, the tumor was deemed to be smaller than 2 cm. When several medical imaging procedures were identified within this period, we included the most reliable imaging procedure available among 1) duodenopancreatic endoscopy ultrasound (EUS), 2) MRI (Magnetic Resonance Imaging) and 3) tomodensitometry. Th-NET occurrence was also taken into account, given its pejorative impact on survival (8). As these tumors may have occurred later during the patients' follow-up (after DP-NET diagnosis) and as new secreting or non-secreting DP-NET may also be identified later and/or prove to be larger, we considered these variables as time-updated covariates in the statistical analyses. DP-NETs secreting glucagon and VIP were too rare to be included in the statistical analyses. The impact of treatments were not taken into account in the study (surgery, chemotherapy, targeted therapies, radiotherapy) as we were interested by assessing the prognostic effect of the variable of interest in the context of standard care and not by the impact of the timing and the type of treatment.

Statistical analysis

Descriptive results are expressed as percentages for qualitative variables and as means or medians (with standard deviations or inter-quartile ranges) for continuous variables, according to their distribution.

The baseline data (time zero) was the date of the DP-NET diagnosis. In the first analysis, time to events was defined as the interval between DP-NET diagnosis and death. Subjects who did not die were censored at their last follow-up. The probability of death was estimated using the Kaplan-Meier method and compared using log-rank tests. Factors associated with death were identified using a Cox proportional hazards model including metastasis occurrence as a time-updated variable.

In order to investigate factors specifically associated with metastasis occurrence 1) metastasis free-death on the one hand and 2) death after metastasis occurrence on the other hand, we applied the multi-state Markov piecewise constant intensities model (MKVPCI; 20). Similar to other Markov multi-state models, the MKVPCI model generalizes the single-endpoint survival models to allow *simultaneous* estimation of the effects of prognostic factors on the hazard of transitions between all clinically relevant health states. Accordingly, a single MKVPCI analysis of the entire study cohort allowed us to estimate the separate associations of prognostic factors with each of the events of interest, including the two mutually exclusive events (e.g. metastasis vs metastasis-free death) and events that follow metastasis occurrence (e.g. death after metastasis) (20). The MKVPCI model has been previously shown to be able to separate the effects of prognostic factors on cancer progression from their effects on mortality, both in the analyses of real-life cancer progression studies (21) and in simulations (22). Furthermore, simulations also showed that this model avoids potentially important biases due to non-random censoring (22).

In our study three states were considered: 1) alive without metastases, 2) alive with metastases and 3) death (**figure 2**); this latter state was considered as absorbent. The multi-state MKVPCI model allowed us to estimate separate effects of each prognostic factors on 1) metastases occurrence (transition 1–2), 2) death without metastasis (transition 1–3), and 3) death after a metastasis occurrence (transition 2–3). This piecewise-constant intensities model assumed different intensities (*i.e.* hazard rates) 1) in the first five years of follow-up, and 2) after the fifth year of follow-up, with constant hazard within each of the two time intervals. In the estimation process, a patient who had metastases (*i.e.* transitioned from state 1–2) at time t, was no longer considered at risk of transition 1–3 after, but was then considered at risk of transition 2–3, *i.e.* of death after metastasis. In contrast, patients who died without the identification of any metastasis and, thus, whose first transition was 1–3,

were considered at risk of developing metastases (transition 1–2) until that time, but had their follow-up terminated at time of death, and were not at risk of transition 2–3.

For all analyses, a p-value below 0.05 was considered significant. SAS software version 9.4 (SAS Institute Inc., Cary, USA) and MKVPCI program (20) were used for analyses.

RESULTS

In March, 2015, the GTE MEN1 cohort included a total of 1,286 patients. From these patients, we selected subjects with DP-NETs diagnosed since 1990 and for whom the follow-up was known (n=603; **figure 1, table 1**). Overall, 213 patients had neither family history nor genetic testing. There were 118 patients whose DP-NET was larger than 2 cm (19.6%) at diagnosis, 178 patients with ZES (29.5%), and 70 patients with insulinoma (11.6%). Glugagon secretion was found in 19 (1.5%) cases and VIP secretion in 5 (0.4%) cases. No somatostatin secretion was found. All DP-NETs larger than 2cm were located in the pancreatic gland and were never found in the duodenal wall.

Patients were 41 years old (± 14.9 years) at the time of the DP-NET diagnosis. On average, DP-NETs were diagnosed 2.8 years ± 5.6 years after confirmation of MEN1 diagnosis. The DP-NET diagnosis preceded the MEN1 diagnosis by 22 years in one case and was made up to 27 years after the diagnosis of MEN1 in another case. The overall median follow-up after the diagnosis of DP-NETs was 6.9 years (Inter-Quartile Range [IQR]=3.3-12.0 years). The great majority of patients had only one NET definitely confirmed as DP-NET at time of diagnosis (89.4%), 9.1% had two DP-NETs (25 at baseline, and 39 during their follow-up in a median delay of 43 months [IQR=15-105 months]) and 1.5%, three consecutive DP-NETs (3 patients at baseline, and 6 during their follow-up in a median delay from baseline to the last tumor diagnosis of 170 months [IQR=138-224 months]). Median age at death was 55.5 years (IQR=40.8-66.7).

Causes of death and probability of death, according to patients' characteristics, are presented in **Table 1&2**. Using log-rank tests, patients aged over 40 years, with th-NET or with a mutation in the JunD domain as well as patients with a DP-NET over 2 cm had higher probabilities of dying. The multivariable Cox model confirmed that patients aged over 40 years had shorter survival and as

expected, it showed the major impact of metastasis on the risk of death (Hazard Ratio [HR]=6.25; 95%-Confidence Interval [CI]=3.77-10.35; **Table 3**). Men also tended to have a poorer prognosis than women (HR=1.60; 95%-CI=0.96-2.67; **Table 3**). After adjusting for these factors, ZES- patients had a significant lower risk of death (HR=0.47; 95%-CI=0.27-0.83). Interestingly, testing the interaction between metastasis occurrence and the other covariates revealed that the presence of a ZES had no significant impact on survival in patients without metastasis. In addition, in patients with ZES the occurrence of a metastasis has a much lower impact on survival HR=2.08(95%-CI=0.89-4.87) than in patients without ZES ((HR=12.37 (95%-CI=6.60-23.20)(p for interaction = 0.0005).

The Markov multistate model revealed that accounting for a change in baseline transition intensities before and after 5 years of follow-up improved the model fit (p for the LR Test < 0.001), showing the importance of accounting of these two periods. Indeed, this model showed that the transition intensity towards metastasis occurrence was lower after 5 years, than before. In addition, this model allowed us to identify factors specifically associated with the risk of metastasis occurrence. It showed that the presence of a ZES- (HR=4.36; 95%-CI=2.44-7.77), a tumor larger than 2 cm (HR=2.96; 95%-CI=1.43-6.14), and age over 40 years (HR=1.02; 95%-CI=1.01-1.04) increased the risk of metastasis occurrence (**Table 4**). Men also had marginally higher risk of metastasis development (p=0.0506). The multi-state model also allowed us to estimate the impact of covariates on death after metastasis occurrence. Patients with a tumor larger than 2 cm also had an increased risk of death after metastasis occurrence as did patients over 40 years old and men. Interestingly, the model revealed that insulinoma patients had a lower risk of death after metastasis occurrence (HR=0.15; 95%-CI=0.05-0.48). However, all six patients concerned underwent surgery soon after the metastasis was discovered (median delay=4 months; IQR=0.4-7.0 months). When looking at the patients who died from the metastatic evolution of their endocrine tumor, only five (16%) died when a ZES-DP-NET was present whereas 15 (47%) died when a large DP-NET was present . The only metastatic patient with ins-DP-NET who died also had a 67-mm tumor secreting glucagon.

Finally, as expected, the multistate model showed the poor prognosis of patients with th-NET, especially patients without metastasis (HR=7.45; 95%-CI=1.98-28.06)(**Table 4**). This increased risk of death in patients with th-NET was no longer significant (p=0.1640) when they also had metastasis

from their DP-NET. However, when we tested whether the risk of death associated with th-NET differed according to DP-NET metastasis occurrence, no significant difference was found (p=0.2658) in the Cox model or using the multi-state Markov model. The analysis of the seven th-NETs patients who died (**Table 5**) confirmed that only one case died from the distant metastatic evolution of th-NET. Most of them died from respiratory complications due to loco-regional spread or from other unrelated causes.

DISCUSSION

DP-NETs are responsible for the majority of MEN1-cancer-related deaths (8,9). The indication for surgery in order to prevent metastatic spread and/or to improve survival remains a difficult issue since various kinds of DP-NETs may occur synchronously or metachronously during the life span of MEN1 patients. This study provides for the first time a new insight on this issue. It included a large cohort of 603 MEN1 patients with DP-NETs, whose diagnosis was made after 1990 in order to compare patients evaluated with the same imaging procedures. Nevertheless despite the large size of the cohort a small number of prognostic factors were evaluated because of limited statistical power due to the number of events (neuro endocrine distant metastasis occurrence or death). Moreover neither adrenal tumors, which are usually benign, nor DP-NETs secreting Glucagon (n=19), or VIP (n=5), which are very rare, were taken into account. Surgery or other treatments were not taken into account in the study as we intended to estimate the impact of patients and DP-NETs characteristics available from standard workout before any treatment were decided. In addition surgeries were carried out for different intricate and successive purposes (secretion control, cancer prevention, cancer treatment)(347 surgeries among 287 patients) which heterogeneity prevent to correctly model the effect of such an information. Nevertheless, this multicenter study based on the largest MEN1 cohort has the advantage of limiting selection bias and provides useful conclusions that may help to indicate surgery according to simple and independent parameters.

Cox's model is the most popular method for analyzing survival data. This model confirmed that occurrence of metastases was the major event associated with shorter survival. The Cox model allowed us to determine whether the effects of factors associated with the risk of death changed after metastasis occurrence by testing the interaction between each factor and metastasis. Nevertheless, this model is limited since we were particularly interested in identifying factors associated with a higher risk of metastasis occurrence, while accounting for the risk of death (**Table 3**). Therefore, the multistate Markov model was used to address this specific issue.

First of all, it appeared clearly that the presence of any tumor larger than 2 cm located in the pancreatic gland whatever the type of secretion was associated with an increased risk of metastasis occurrence. Large tumors were also responsible for shortened survival. Until now the impact of the size (>2 cm) of the largest DP-NET on the occurrence of synchronous, metachronous metastases and survival has only been studied in cases of DP-NET without associated endocrine secretion (NF-DP-NETs)(13-16). In these cases, the GTE group and the Dutch MEN1 Study Group have already advised surgery (13-16). More than fifteen years ago, surgery has been also advocated among ZES patients when a large NET was visible (without knowing what exactly was its endocrine status and implicitely associating this NET to a secreting gastrinoma responsible for ZES) (11). In this first study, a relationship between large size of associated NETs (>3 cm) and metachronous metastases occurrence was found. Nevertheless this paper was only focused on ZES patients and no survival study was carried out. Indeed, the present study indicates that the “2cm size–rule” may now be generalized to any MEN1 patients with large DP-NETs independently of their endocrine secretory status. The presence of large NETs significantly increases not only the metastatic risk but also consequently decreases survival. This conclusion is of paramount importance since MEN1 patients may display various secreting and non-secreting DP-NETs together, and it is difficult to know which one is responsible for a particular secretion.

Secondly, ZES were proved to be associated with an increased risk of distant metastases and this, regardless of the size of the tumor. This observation confirms that ZES-DP-NETs are highly likely to metastasize *“per se”* (10). ZES-DP-NETs’ natural history may be compared to the natural history of sporadic duodenal or small bowel NETs associating small primaries with numerous, larger

nodes and liver metastases Recent studies demonstrate that MEN1 related ZES-DP-NETs (gastrinomas) are located in the duodenum (10,23,24). The possibility of primary lymph node gastrinoma has also been raised. Nevertheless, a NIH study showed that primary lymph node gastrinomas may occur up to 10% in case of sporadic ZES but was never encountered in case of MEN1 disease (25). Most of the time, MEN1 related ZES are due to small and multiple duodenal DP-NETs primaries (gastrinomas) possibly associated with numerous large liver metastases (24).

By contrast to large DP-NETs, metastatic ZES-DP-NETs were not significantly associated with a higher risk of death in the multi-state Markov model. The comparison may be made with sporadic NETs since five-year survival has been evaluated at 54% in cases of sporadic metastatic small bowel NETs versus only 19.5% in cases of sporadic metastatic DP-NETs (26,27). As well, a median survival of 57 months was found in cases of sporadic metastatic duodenal NETs versus only 24 months in cases of sporadic metastatic DP-NETs (28).When looking specifically at outcomes in the 38 metastatic ZES-DP-NET patients, it appeared clearly that only five of them died from their metastases. Moreover, the Cox model adjusted for the metastatic status of patients showed that ZES-DP-NETs patients were at a lower risk of death. Indeed, both the Cox and Markov models are useful to understand the natural history of MEN1-related DP-NETs. In patients with metastasis, the reasons why ZES-DP-NETs do not seem to significantly decrease survival may be due to medical and surgical treatments. In the NIH experience, patients with advanced tumors who underwent surgical resection for ZES-DP-NETs had the same survival as patients with limited disease and those without identifiable tumors (23). Another explanation may be related to different forms of ZES-DP-NETs. The same NIH group already pointed out two different forms of MEN1-related ZES-DP-NET: an aggressive and a non-aggressive form (10). The authors found a majority of 49 cases (86%) of non-aggressive forms versus only eight cases (14%) of aggressive forms. The majority of patients with better survival harbored non-aggressive forms, even those with associated metastases. Finally, aggressive tumor growth was associated with significantly shorter survival conversely to liver metastases without aggressive tumor growth. Five-year survival in patients with aggressive disease was 88% (95%-CI=53-98), whereas it was 100% (95%-CI=92-100) in patients with non-aggressive disease with or without metastases (p=0.0012). Therefore, operating on ZES-DP-NETs in order to

increase survival remains a very controversial issue. Nevertheless, surgery is recommended when ZES-DP-NET is proved to be located in the pancreas and larger than 2cm (very rare condition only definitely confirmed after surgical resection, specimen analysis, and ZES cure) or if ZES is associated with another large NET located in the pancreas, usually not ZES-related, since tumor size is an independent prognostic factor. Therefore, it becomes more appropriate to use the term "ZES-DP-NET (gastrinoma) associated with a large DP-NET" instead of "large ZES-DP-NET" (11). Further research is required to stratify aggressive versus less aggressive ZES-DP-NETs. A recent study shows that ^{18}F-FDG PET/CT was an effective screening modality for the identification of MEN1 related DP-NETs with high malignant potential (29). The authors concluded that surgical resection is recommended for FDG-avid DP-NETs. Nevertheless, this interesting study has several limitations since the correlations between FDG-PET imaging and the Ki67 index were evaluated in only seven patients, whose tumors were 2 cm large or more in six of the seven cases. Unfortunately, ZES-DP-NETs are usually small, numerous and located in the duodenum, and we demonstrated here that they can metastasize independently of their size. Therefore, the right imaging and prognostic tool is still lacking. We can imagine that radical resections, such as Whipple procedures, might be reserved in the future for the subgroup of patients harboring aggressive ZES-DP-NETs i.e. probably 10 to 20% of the cases according to NIH data.

Thirdly, ins-DP-NETs were not likely to metastasize. Among 70 patients, only six developed liver or lung metastases and only one of them died. Moreover, this patient carried both an ins-DP-NET and a large glucagon-secreting DP-NET. Paradoxically, among patients with metastasis, the quite rare individuals with ins-DP-NETs had a significantly lower risk of death. Indeed, ins-DP-NETs are an indication for surgery as soon as possible in order to control insulin secretion. Therefore, it seems very difficult to interpret the possible favorable effect of the surgical treatment on survival in this very small group of six patients.

Finally, the multi-state Markov model showed that patients with th-NETs died from their primary disease without going through the distant metastasis stage. The location of th-NETs in the mediastinum and their rapid growth may explain why loco-regional consequences were the main causes of death (8,30).

We thus concluded that among factors that could be associated with metastasis occurrence in MEN1 patients with DP-NETs, the size of the largest DP-NET, usually located in the pancreas, and the presence of a ZES-DP-NET, usually located in the duodenum, numerous and of little size, are the main independent risk factors. Large tumors increased the risk of metastatic spread and decreased survival after metastasis occurrence whatever the associated neuroendocrine secretion. ZES-DP-NETs may metastasize but do not seem to significantly decrease overall survival. Nevertheless, aggressive forms with a worse prognosis do exist (10). They may be associated with particular types of mutations, such as mutations which affect the JunD domain or its partners, despite the fact that we were not able to demonstrate such an interaction in our study (19). Investigating prognosis factors such as Ki67 index obtained by FNA, as micro RNA or circulating tumors cells seems required in the future (31). Finally, indications for surgery for MEN1-related DP-NETs may rely on several simple independent criteria as long as the patient is in good overall health with a resectable tumor: 1) as already known in any case of non-ZES functional lesions (e.g. insulinoma, glucagonoma, VIPoma) irrespective of size, 2) in any case of DP-NETs reaching 2 cm in size to prevent metastases and to increase survival irrespective of secretion and 3) maybe in cases of ZES-DP-NETs if the tumor harbors aggressive features using a surgical technique which still needs to be evaluated.

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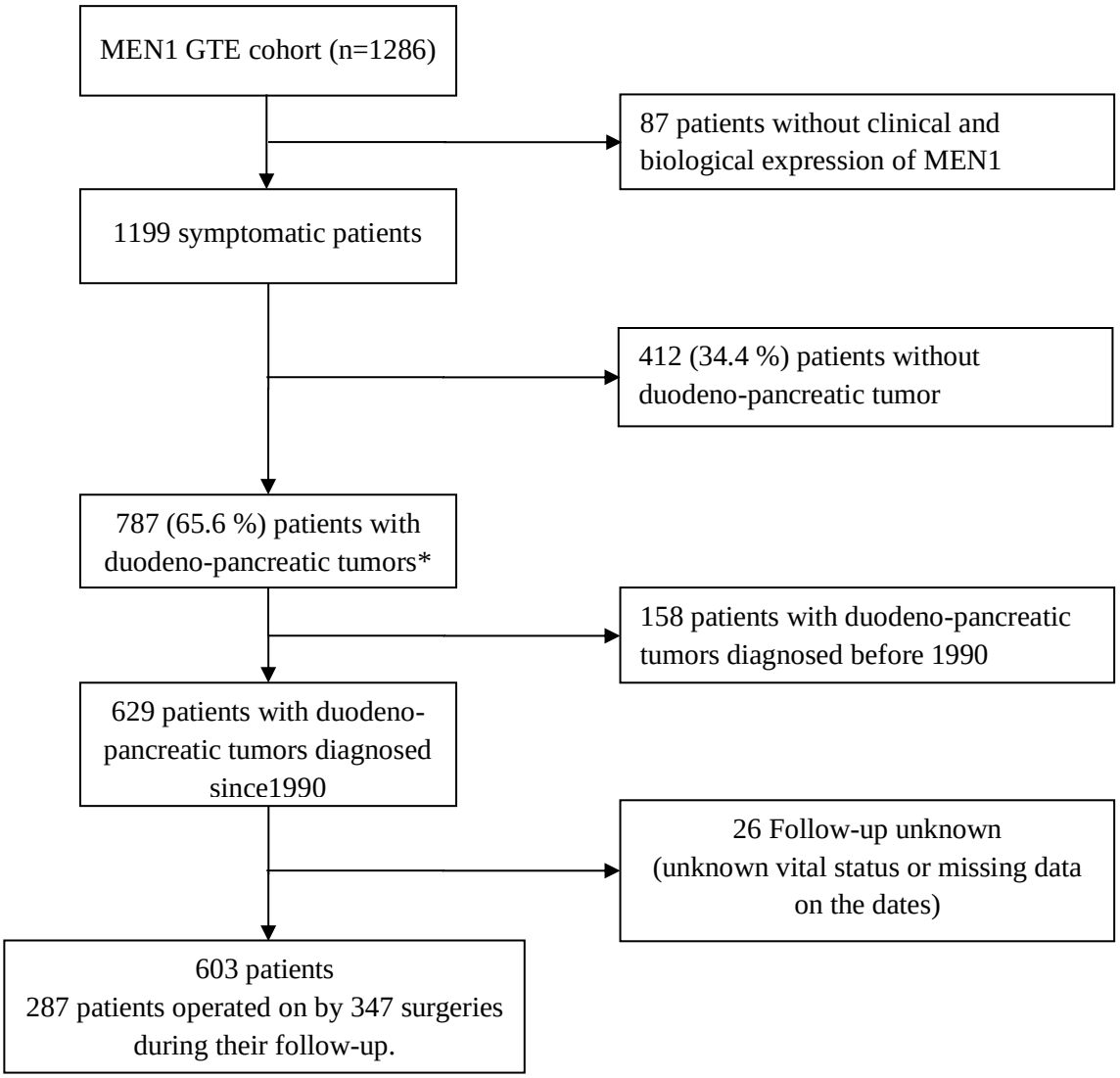
Figure 1.

Flow chart



*DP NETs were diagnosed using various techniques: 1- MRI or CT-scan confirmed by specific imaging studies (scintigraphy)(n=160), 2- histology obtained by: a- FNA during Endoscopy Ultrasound Sonography (EUS) or by resection using gastroduodenal endoscopy (n=64) b- by surgery (n=247). 3- Abnormal endocrine secretions (gastrin, insulin) since they were related to the presence of ZES-DP-NET or ins-DP-NET even though the precise location of the NETs could not always be found (n=248).

Figure 2.
Three-state distant metastases-death model (NEM1 GTE cohort)

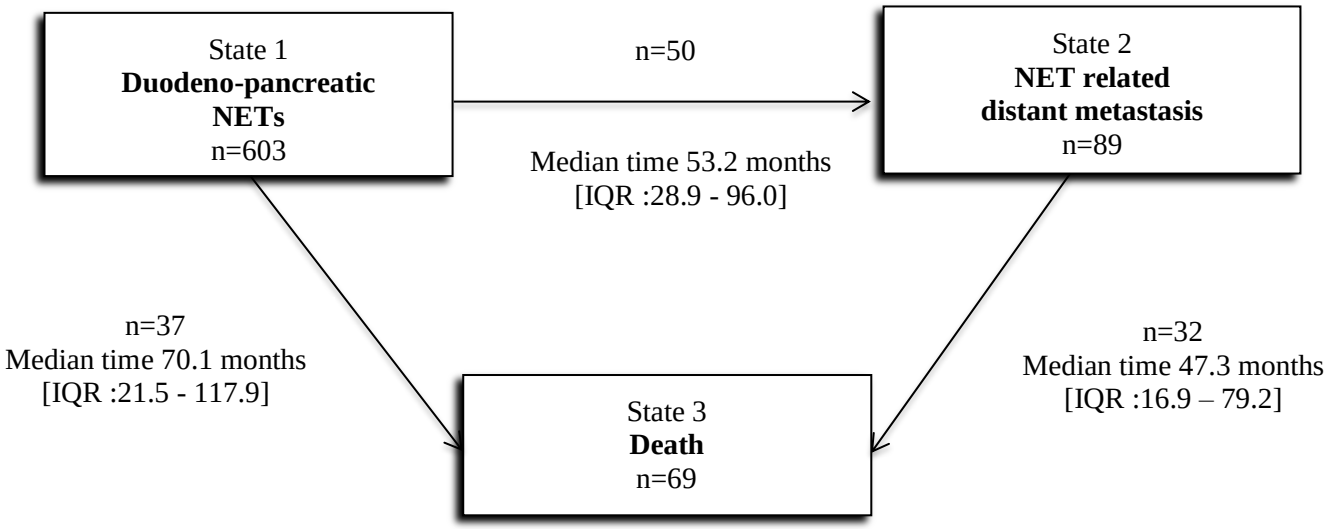


Table 1. Patients' characteristics at the DP-NET diagnosis and death probabilities according to these characteristics (MEN1 GTE cohort)

	All n=603		Death n=69		Death probabilities [95%-CI]			
	n	(%)	n	(%)	2 years	5 years	8 years	p-value
Age								
< 40y	299	(49.6)	17	(5.7)	1.5% [0.6 - 3.9]	2.4% [1.1 - 5.3]	5.6% [3.2 - 9.7]	< 0.0001
≥ 40y	304	(54)	52	(17.1)	3.6% [1.9 - 6.5]	9.8% [6.6 - 14.3]	17.7% [13.0 - 23.8]	
Gender								
Males	277	(45.9)	39	(14.1)	3.1% [1.6 - 6.1]	7.8% [4.9 - 12.1]	15.1% [10.7 - 21.0]	0.0563
Females	326	(54.1)	30	(9.2)	2.0% [0.9 - 4.5]	4.6% [2.6 - 8.0]	8.6% [5.5 - 13.3]	
Pancreatic tumor secretion								
ZES-DP-NETs								
Yes	178	(29.5)	24	(13.5)	1.9% [0.6 - 5.7]	4.7% [2.3 - 9.7]	9.7% [5.7 - 16.2]	0.8669
No	425	(70.5)	45	(10.6)	2.8% [1.5 - 5.0]	6.7% [4.5 - 10.0]	12.6% [9.1 - 17.2]	
Ins-DP-NET								
Yes	70	(11.6)	6	(8.6)	0%	0%	4.4% [1.1 - 16.5]	0.2630
No	533	(88.4)	63	(11.8)	2.8% [1.7 - 4.7]	6.8% [4.8 - 9.7]	12.6% [9.5 - 16.6]	
Thymic tumors								
Yes	31	(5.1)	7	(22.6)	13.4% [4.5 - 36.0]	13.4% [4.5 - 36.0]	22.8% [10.2 - 46.4]	0.0059
No	572	(94.9)	62	(10.8)	2.1% [1.2 - 3.7]	5.8% [4.0 - 8.4]	11.1% [8.3 - 14.7]	
JunD genotype								
Yes	196	(32.5)	25	(12.8)	4.5% [2.3 - 8.8]	7.2% [4.1 - 12.4]	12.2% [7.8 - 18.8]	0.0122
No	340	(56.4)	30	(8.8)	1.3% [0.5 - 3.3]	4.5% [2.6 - 7.8]	10.0% [6.6 - 14.9]	
Not available	67	(11.1)	14	(20.9)	3.2% [0.8 - 12.1]	11.0% [5.1 - 23.0]	19.1% [10.2 - 34.2]	
Tumor size at DP-NET diagnosis								
< 2cm	316	(52.4)	20	(6.3)	0.8% [0.2 - 3.0]	3.5% [1.8 - 6.8]	8.3% [5.1 - 13.4]	0.0088
> or = 2cm	118	(19.6)	16	(13.6)	4.7% [2.0 - 10.9]	9.2% [4.9 - 17.0]	15.5% [9.1 - 25.7]	
Not available	169	(28.0)	33	(19.5)	4.2% [2.0 - 8.7]	8.5% [5.0 - 14.3]	14.4% [9.5 - 21.6]	
Metastasis								
No	514	(85.2)	37	(7.2)	-	-	-	-
At DP-NET diagnosis	39	(6.5)	16	(41.0)				
Occurrence during the follow-up	50	(8.3)	16	(32.0)				

Table 2: Causes of death among the 603-GTE cohort of patients with DP-NETs

Causes of death (∑=67)*	**Related to NET (N=46)**	**Non NET cancer related (N=8)**	**Medical causes (N=13)**
Not related to distant metastatic spread (N=31)	Operative deaths Septicemia (n=1) Mesenteric ischemia (n=1) Pancreatitis (n=1) Hemorraghe (n=1) Thymic loco regional spread (n=4) Ulcerous disease (n=2) Diabetes post total pancreatectomy (n=1) Metabolic disorders (ZES) (n=1) Chemo embolization (n=1) Pituitary carcinoma (n=1)	Leukemia (n=4)	Alcohol (n=3) Cardiac insufficiency (n=1) Cardiac infarction (n=1) Pulmonary embolism (n=3) Renal insufficiency (n=1) Angiocholitis (n=1) Aspergillosis (n=1) Stroke (n=2)
Related to distant metastatic spread (N=36)	Pancreas (n=26) Bronchus (n=3) Thymus (n=1) Adrenal carcinoma (n=1) Liposarcoma (n=1)	Lung (n=2) Tongue (n=1) Pancreatic adenocarcinoma (n=1)	

*Two missing cases died from unknown cause (not related to distant metastatic NET disease)

Table 3. Factors associated with death (Cox model; MEN1 GTE cohort; n=603)

			Model with interaction gastrin secretion*metastasis	
	Hazard Ratio [95%-CI]	p-value	Hazard Ratio [95%-CI]	p-value
Age ≥ 40 yrs vs < 40 yrs	3.28 [1.85 – 5.81]	< 0.001	3.56 [1.98-6.40]	< 0.001
Men vs women	1.60 [0.96 – 2.67]	0.071	1.50 [0.89-2.51]	0.125
ZES-DP-NET (yes vs no)	0.47 [0.27 – 0.83]	0.008	-	
ins-DP-NET (yes vs no)	0.67 [0.28 – 1.59]	0.363	0.84 [0.35-2.04]	0.703
Thymic tumors (yes vs no)	1.95 [0.83 – 4.57]	0.125	2.33 [0.98-5.57]	0.057
JunD genotype (yes vs no)	1.30 [0.76 – 2.23]	0.342	1.28 [0.74-2.20]	0.374
Size ≥ 2 cm (yes vs no)	1.68 [0.86 – 3.26]	0.127	1.51 [0.77-2.95]	0.233
Metastasis	6.25 [3.77 – 10.35]	< 0.001	-	
Interaction metastasis-gastrin secretion (reference=no ZES-DP-NET- no metastasis):				
No ZES-DP-NET – Metastasis	-		12.37 [6.60-23.20]	< 0.001
ZES-DP-NET – No metastasis	-		1.08 [0.54-2.14]	0.830
ZES-DP-NET – Metastasis	-		2.08 [0.89-4.87]	0.090

Interaction with metastasis was tested for all the covariates. This interaction was significant only for ZES-DP-NETs (p-value < 0.001)

Table 4. Prognostic factors of metastasis and death, either without metastasis or after metastasis occurrence, in patients with DP-NETs (MKVPCI Multistate Markov model; NEM1 GTE Cohort, n=603)

	Transition 1-2 (Metastasis)		Transition 1-3 (Death)		Transition 2-3 (Metastasis-Death)	
	Estimation [95%-CI]	p-value	Estimation [95%-CI]	p-value	Estimation [95%-CI]	p-value
Age ≥ 40 yrs vs < 40 yrs	1.02 [1.01 – 1.04]	0.0088	0.97 [0.89 – 1.05]	0.4041	1.01 [1.00 – 1.02]	0.0276
Men vs women	1.72 [1.00 – 2.96]	0.0506	0.78 [0.25 – 2.46]	0.6762	2.63 [1.26 – 5.48]	0.0096
ZES-DP-NET (yes vs no)	4.36 [2.44 – 7.77]	< 0.0001	0.48 [0.13 – 1.74]	0.2657	0.58 [0.28 – 1.21]	0.1466
Ins-DP-NET (yes vs no)	1.18 [0.50 – 2.83]	0.7054	0.63 [0.14 – 2.71]	0.5323	0.15 [0.05 – 0.48]	0.0012
Thymic tumors (yes vs no)	0.81 [0.23 – 2.91]	0.7499	7.45 [1.98 – 28.06]	0.0030	2.28 [0.71 – 7.25]	0.1640
JunD genotype (yes vs no)	1.22 [0.71 – 2.12]	0.4709	1.69 [0.55 – 5.17]	0.3582	1.13 [0.60 – 2.14]	0.7012
Size ≥ 2 cm (yes vs no)	2.96 [1.43 – 6.14]	0.0035	0.63 [0.06 – 6.18]	0.6903	3.26 [1.36 – 7.76]	0.0078

Table 5. Characteristics and causes of death of MEN1 patients with thymic tumors among the 603-GTE cohort of patients with DP-NETs

N°	Sex	Year of MEN11 diagnosis	Year of thymic NET diagnosis	Age at thymic NET diagnosis	Age at death	Surgical resection	JunD LOI*	Histology	Cause of death
910	H	1999	2003	38 years	43 years	Yes	No	Low grade NET	Metastatic evolution of a pancreatic NET.
258	F	1995	2013	32 years	36 years	Yes	No	AC	Pneumocystis. Respiratory Insufficiency.
286	H	1995	1995	50 years	59 years	Yes	Yes	AC	Metastatic evolution of the thymic NET.
455	H	1994	1994	49 years	59 years	Yes	No	NEC	Respiratory Insufficiency. Superior Vena cava Syndrome.
267	H	1994	1990	41 years	51 years	Yes	No	AC	Metastatic evolution of a pancreatic NET.
338	H	1994	1994	46 years	58 years	No	No	AC	Pleuresia. Respiratory Insufficiency.
360	H	1987	1986	36 years	45 years	Yes	Yes	AC	Respiratory Insufficiency.

NET Neuro Endocrine Tumor ; NEC Neuro Endocrine Carcinoma : LOI Loss Of Interaction ; AC Atypical carcinoid