Prognostic value of WHO grade in pancreatic neuro-endocrine tumors in Multiple Endocrine Neoplasia type 1: Results from the DutchMEN1 Study Group

Elfi B. Conemans a, b, Lodewijk A.A. Brosens c, Gabriela M. Raicu-Ionita c, Carolina R.C. Pieterman b, Wouter W. de Herder d, Olaf M. Dekkers c, Ad R. Hermus f, Anouk N. van der Horst-Schrivers g, Peter H. Bisschop h, Bas Havekes i, Madeleine L. Drent j, H. Th Marc Timmers k, G. Johan Offerhaus c, Gerlof D. Valk b, Menno R. Vriens a,*

* Corresponding author. University Medical Center Utrecht, Department of Endocrine Surgery, Mailbox G04.228, PO Box 85500, 3508 GA Utrecht, The Netherlands. E-mail address: mriens@umcutrecht.nl (M.R. Vriens).

A R T I C L E   I N F O

Article history:
Received 6 January 2017
Received in revised form 28 July 2017
Accepted 30 July 2017
Available online 31 July 2017

Keywords:
Multiple endocrine neoplasia type 1
Pancreatic neuroendocrine tumors
Liver metastases
WHO grade
Prognosis

A B S T R A C T

Background: The prognostic value of WHO grade in pancreatic neuroendocrine tumors (PanNETs) in patients with Multiple Endocrine Neoplasia Type 1 (MEN1) is unknown.

Methods: We performed a cohort study using the Dutch National MEN1 database, which includes >90% of the Dutch MEN1 population with data collected between 1990 and 2014. Formalin-fixed paraffin-embedded tissue blocks from the largest resected PanNET per patient were collected. MIB1 staining was performed and KI67 labeling index (LI) was determined by manual eye-counting under a microscope and by digital image analysis. Mitotic count was evaluated from hematoxylin & eosin stains. Association between WHO grade and (time until) development of liver metastases was calculated.

Results: Sixty-nine MEN1 patients who underwent pancreatic surgery were included. Ten patients (14%) developed liver metastases and all had PanNETs ≥3 cm. WHO G1, G2 and G3 PanNETs were seen in 83% (n = 57), 16% (n = 11) and 1% (n = 1) respectively. In non-functioning PanNETs >2 cm, liver metastases occurred in 80% of WHO G2 PanNETs (4/5) compared to 23% (5/22) in WHO G1 PanNETs (p = 0.03) when WHO grade was based on mitotic count only. This significant association was not seen for WHO grade based on KI67 LI. After five years, liver metastases in non-functioning PanNETs were not seen in tumors ≥2 cm, in 10% of the large WHO G1 (according to mitotic count only) tumors and in 60% of large WHO G2 tumors (p-value 0.000).

Conclusion: High mitotic count is correlated with poor prognosis in MEN1 patients with large non-functioning PanNETs.

© 2017 IAP and EPC. Published by Elsevier B.V. All rights reserved.

Introduction

Multiple Endocrine Neoplasia type 1 (MEN1) is an autonomic dominat heritable tumor syndrome. The disease is caused by
germline mutations in the MEN1 gene located on chromosome 11q13 encoding the tumor suppressor protein menin, which has a role in epigenetic control of gene expression [1,2]. The three main clinical manifestations are parathyroid adenomas leading to primary hyperparathyroidism, neuroendocrine tumors of duodenum and pancreas and pituitary adenomas. Duodenopancreatic NETs occur with an estimated prevalence of 30–80% and are often multiple [2]. Progressive duodenopancreatic NETs are considered to be the most frequent cause of death in MEN1 patients [3–5].

Functioning pancreatic NETs (PanNETs) cause clinical syndromes due to excessive production of hormones such as insulin, glucagon, somatostatin and vasoactive intestinal peptide, whereas non-functioning tumors are defined as PanNETs without a clinical syndrome. Gastrin producing NETs in MEN1 causing Zollinger-Ellison Syndrome originate predominantly in the duodenum and pancreatic gastrinomas are rare [6].

The only curative therapy for PanNETs is early surgical resection. Timing and extent of surgery for MEN1-related PanNETs are a matter of debate. Compared to their sporadic counterparts, PanNETs in MEN1 occur at a younger age, are often multiple, may be detected at an earlier stage due to screening programs focused on detection of PanNETs and may occur in the presence of comorbidity related to tumors in other organs. According to current MEN1 guidelines patients with functioning PanNETs (apart from gastrinomas), PanNETs > 1 cm and rapid progressive PanNETs are being operated on [7]. Besides symptom control in case of functioning PanNETs, the primary goal in PanNET treatment is to prevent metastatic disease. After surgery, MEN1 patients are followed intensively for many years in order to identify tumor recurrence and/or progression at an early stage. Currently it is not possible to identify MEN1 patients at risk for adverse events such as metastatic disease after PanNET resection. Improved understanding of prognostic factors is needed for optimization (individualization) of MEN1-related PanNET treatment. In sporadic PanNETs WHO grade based on Ki67 labeling index (LI) and mitotic count have been shown to be of prognostic value [8,9], but no data are available for MEN1-related PanNETs. Therefore, the aim of this study is to analyze the prognostic value of WHO grade in surgically resected MEN1-related PanNETs.

Methods

Study design

Patients were selected from the longitudinal Dutch national Database of the DutchMEN1 Study Group (DMSG) comprising > 90% of the Dutch MEN1 population. All MEN1 patients included in this database were diagnosed according to clinical practice guidelines [7], were aged 16 years and older, and were under treatment in one of the university medical centers (UMCs) in The Netherlands. Identification of patients was done by standardized selection procedures using the hospital registry system. Data were collected retrospectively every quarter from 1990 until 2013 using a predefined protocol and decision rules for registration of data. MEN1 patient data collected before 1990 were also available, but not collected longitudinally. The Medical Ethical Committees of all UMCs in the Netherlands approved the study protocol for data collection. The DMSG database has been described in detail before [10,11]. Clinical data used in this study are extracted from the DMSG-database.

Patient- and tumor selection

Patients who underwent pancreatic surgery for PanNET(s) were selected and the pathology reports of these patients were evaluated. The largest PanNET from each patient was identified in case of multiple PanNETs. Tissue slides and/or formalin-fixed paraffin-embedded (FFPE) tissue blocks were collected from archives of Pathology departments throughout the Netherlands in collaboration with “the nationwide network and registry of histology and cytopathology in the Netherlands” (PALGA) [12]. Patients were excluded from this study when FFPE tissue blocks from the largest PanNET were not available.

Definition of an insulinoma is a positive 72 h prolonged supravisceral fast prior to resection of the PanNET that was included in this study. We did not register glucagonomas, VIPomas or somatostatinomas in our database. PanNETs not meeting the criteria for insulinoma, were classified as non-functioning PanNETs. The reported prevalence of glucagonomas, VIPomas and somatostatinomas in the literature is low (1.6%, 1% and 0.65% respectively) [13]. Surgical procedures were classified into enucleation, distal pancreatectomy, Whipple/Pylorus-Preserving Pancreatocoduodenectomy (PPPD), total pancreatectomy or a combination of these strategies. Treatment with somatostatin analogue (SSA) for at least six months from PanNET resection until the end of follow up was recorded.

Our study was performed according to national guidelines with respect to the use of ‘excess tissue’ and ethical approval for this study was obtained from the scientific committee of PALGA and of the Medical Ethical Committee of the UMC Utrecht.

Pathological characteristics

MIB1 staining was performed on FFPE tissue slides according to standardized protocols using the automated IHC staining system Ventana Bench Mark ultra. The Ki67 LI was determined in the areas of highest labeling (hot spots) by manual eye-counting under a microscope of MIB1 positive cells among 2000 cells by two experienced pathologists independently (GJO and GMR). In addition, Ki67 LI was determined in hot spots by digital image analysis. The entire immunostained tissue sections were scanned and hot spots were manually selected for digital quantification of Ki67 LI (PACS, Sectra AB, Linköping, Sweden). After digital examination, an experienced pathologist (LAB) checked whether the positive cells within the selected areas were indeed tumor cells. Ki67 LI was defined as the mean percentage of positive tumor cells within two areas of 1000 cells.

Hematoxylin & eosin stained (H&E) tissue slides were used for counting the number of mitoses per 50 HPF [14]; this number was divided by 5 to obtain the number per 10 HPF. In small tissue samples, proper counting was not possible and these samples were excluded for this part of the analysis.

Mitotic rate was determined by one pathologist (CMR) and two well trained technicians. The pathologists and the technicians were blinded for all clinical data. The results from the two observers regarding Ki67 LI and mitotic count were compared and discrepancies were discussed and re-evaluated.

The following classification was used for WHO grade: G1: Ki67 LI <3 and mitosis <2/10 high power fields (HPF); G2: Ki67 LI 3–20 and/or mitoses 2–20/10 HPF; G3 Ki67 LI >20 and/or mitosis >20/10 HPF [14–16]. In case of discrepancies between WHO grade according to mitotic count and Ki67 LI the highest of the two determines WHO grade. Unless stated otherwise, Ki67 LI throughout this manuscript refers to manual eye-counting.

Tumor size as reported in the pathological reports was used for this study. If no size was reported, H&E slides of the PanNET were checked to see whether the tumor was >2 cm as this is a generally accepted criterion for surgical resection of non-functioning PanNETs [17].
Outcome measures

The main outcome measure in this study is (time to) development of PanNET-related liver metastases. Patients were considered to have liver metastases when [1] this diagnosis was confirmed by pathological examination or [2] radiological examination was positive for liver metastases. Radiology was positive when lesions suspicious for liver metastases were described on consecutive radiological exams (CT- and/or MRI). Each case with liver metastases was evaluated in detail by an expert panel consisting of two endocrinologists (GV and WH) and one endocrine surgeon (MV), all with expertise in treatment of MEN1 patients. This panel decided per case whether liver metastases were PanNET related and which PanNET was most likely causing the liver metastases. The following data were taken into account: date of diagnosis of liver metastases, date of diagnosis and resection of the largest PanNET, presence of other (non) neuro-endocrine tumor(s) and date of diagnosis of these tumor(s). End of follow up was defined as either the time of diagnosis of PanNET-related liver metastases, non-PanNET related liver metastases, death or end of data collection.

Statistical analysis

Patient and tumor characteristics at time of PanNET resection were presented as medians and ranges for quantitative parameters, and as numbers for categorical variables. The Fisher’s exact test, Mann-Whitney U test or independent t-test were used when appropriate and p-values <0.05 were considered significant. Kaplan-Meier survival estimates were used to model follow up for liver metastases and the log-rank test was used to compare time until development of liver metastases between subgroups. Multivariate analysis was not performed due to the limited number of outcomes. Inter-observer variability for WHO grade based on Ki67 LI and mitotic count was assessed by Cohen’s kappa coefficient. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, Ill., USA Version 23.0).

Results

Baseline characteristics

In total 76 patients of the Dutch MEN1 population had undergone pancreatic surgery for PanNET. Paraffin tissue blocks of the largest PanNET were available from 70 patients (92%). One patient with multiple large PanNETs was excluded from this study because it was not possible to assign the liver metastases to a specific PanNETs. Clinical characteristics of the included patients are summarized in Table 1. Median age at PanNET resection was 41 years. Fifteen patients had an insulinoma. One patient (6.7%) in the insulinoma group and nine patients (17%) with non-functioning PanNETs developed liver metastases after a mean follow up from time of 7.8 and 6.6 years, respectively. In total, two patients had synchronous liver metastases and eight patients had metachronous liver metastases. Two patients died of metastatic PanNET and six patients without PanNET-related liver metastases died of other causes (complications pancreatoc PART (n = 1), gastro-intestinal bleeding (n = 1), adenocarcinoma of unknown primary (n = 1), metastatic thymic NET (n = 2), metastatic gastrinoma (n = 1)).

Pathological characteristics

Pathological characteristics are summarized in Table 2. Median tumor size was 2.5 cm [range 0.3–20] and 43% of the PanNETs were ≤2 cm. WHO G1 PanNETs were found in 57 cases (83%), 11 (16%) PanNETs were WHO G2 tumors and one PanNET was WHO G3 when using the standard definition for WHO grade. All PanNETs

Table 1
Clinical characteristics of the MEN1 patients of whom a pancreatic neuroendocrine tumor was included in this study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 69)</th>
<th>Insulinoma (n = 15)</th>
<th>NF-PanNET (n = 53)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>34</td>
<td>3</td>
<td>30</td>
<td>0.018</td>
</tr>
<tr>
<td>female</td>
<td>35</td>
<td>12</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Age at PanNET resection median (range)</td>
<td>41 [20–81]</td>
<td>33 [20–81]</td>
<td>42 [20–69]</td>
<td>0.038</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>enucleation</td>
<td>17</td>
<td>4</td>
<td>13</td>
<td>NA</td>
</tr>
<tr>
<td>distal pancreatectomy</td>
<td>35</td>
<td>8</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>whipple/PPPD</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>total pancreatectomy</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>combination</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>SSA treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>10</td>
<td>1</td>
<td>9</td>
<td>0.439</td>
</tr>
<tr>
<td>no</td>
<td>59</td>
<td>14</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Follow up (years) mean [95% CI]</td>
<td>7.0 (5.5–8.5)</td>
<td>7.8 (4.3–10.8)</td>
<td>6.6 (4.9–8.2)</td>
<td>NA</td>
</tr>
<tr>
<td>Liver metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes, synchronous</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>yes, metachronous</td>
<td>8</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>59</td>
<td>14</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes, due to metastatic PanNET</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>yes, due to other cause</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>61</td>
<td>13</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

PanNET = pancreatic neuroendocrine tumor; NF-PanNET = non-functioning pancreatic neuroendocrine tumor; PPPD = Pylorus-Preserving Pancreatocoduodenectomy; SSA = somatostatin analogue; CI = confidence interval.

a In 1 case it was not clear whether the PanNET was an insulinoma or non-functioning PanNET.

b 95% CI = (−4.4–2.5).
were well differentiated, including the G3 tumor (Ki67 LI 47%). Cohen's kappa coefficients were 0.701 for WHO grade based on Ki67 LI and 0.797 for mitotic count. Discrepancies between WHO grade based on mitotic count and Ki67 LI were seen in eight cases; WHO grade based on Ki67 LI was higher in five of these cases. In eight cases with G2 PanNETs according to Ki67 LI, the labeling indices were as follows: five cases had Ki67 LI 3–5%, two cases 6–10%, one case 11–15%.

Outcome

Liver metastases did not occur in patients with PanNETs ≤2 cm. In our cohort, the smallest PanNET causing liver metastases was 3 cm. The PanNET from the single insulinoma patient with liver metastases was WHO G1 according to both mitotic count and Ki67 LI (data not shown). Among patients with small (<2 cm) non-functioning PanNETs, WHO G1, G2 and G3 tumors were seen in 95% (n = 20), 5% (n = 1) and 0%, respectively (Table 3a). For patients with large (>2 cm) non-functioning PanNETs these numbers were 68% (n = 19), 29% (n = 8) and 4% (n = 1) for G1, G2 and G3 tumors, respectively. Liver metastases were seen in nine (32%) patients with large PanNETs. Liver metastases occurred in five (26%) patients with WHO G1 PanNETs, three (38%) patients with WHO G2 PanNETs and in the one patient with a WHO G3 tumor (p = 0.35) (Table 3a). These numbers were comparable for WHO grade based on Ki67 LI only by manual eye-counting and digital image analysis (Table 3b and c). Interestingly, of the five patients with large non-functioning WHO G2 PanNETs, four patients (80%) developed liver metastases (p = 0.03) (see Table 3d). After 5 years, 90% of the patients with large WHO G1 based on mitotic count only had no liver metastases and this was 40% for patients with WHO G2 PanNETs (Fig. 1, log-rank p-value 0.000).

Discussion

In this study, the prognostic value of WHO grade in surgically resected PanNETs in the well-described Dutch MEN1 cohort was analyzed. We show that WHO grade based on mitotic count only is associated with the development of liver metastases in MEN1 patients with non-functioning PanNETs. Furthermore, we confirm the previously described association between PanNET size and liver metastases as all patients in our study with liver metastases had pathologically confirmed PanNETs of ≥3 cm. To our knowledge, this is the first study describing distribution of WHO grade in PanNETs in a large MEN1 cohort. The DutchMEN1 study group database includes clinical data from >90% of the total Dutch MEN1 population and is therefore a representative database with reduced chance for selection bias. The unique collaboration between pathology departments throughout the country made it possible to collect tissue blocks from all patients who underwent pancreatic surgery for PanNETs. Comparison with data from sporadic PanNETs is challenging, mainly due to heterogeneity among study cohorts. Differences in inclusion criteria, definitions of functioning and non-functioning PanNETs, and outcome measures are the main matters of concern. In studies focusing on sporadic PanNETs, the frequency of G2 and G3 tumors is higher than in our cohort [18]. This might either reflect early detection of PanNETs in MEN1 patients due to screening programs or different biological behavior. It underscores the importance of prognostic studies for MEN1 PanNETs specifically. Overall survival is mostly used as primary endpoint in prognostic studies from PanNETs [19] and therefore it is difficult to compare these data with our results.
However, high mitotic count has been shown before to be associated with disease-free survival in PanNETs, which is in line with our results [20]. Although survival was not an endpoint of our study, it is noteworthy that the patient with a well-differentiated WHO G3 PanNET had rapid progressive disease and survival was much shorter (9 months) than the reported median survival of 43–75 months for well-differentiated high grade, mainly sporadic, PanNETs [21–23]. As far as we know, poorly differentiated PanNETs (neuroendocrine carcinomas) are not associated with MEN1.

Some limitations of our study need to be discussed. First is the retrospective nature of this study. The decision to perform surgery was made by the treating clinicians and the indication for surgery was not clear from our data. Most obvious reasons for surgery could have been functioning PanNETs (insulinomas), large PanNETs and fast growing PanNETs. Furthermore, a causative relation between the PanNET included in this study and the liver metastases could be argued. Tissue of liver metastases was not available in most cases making it impossible to ascertain the origin of the metastases. To overcome this issue in the best possible way we included only the largest PanNET per patient as it is generally accepted that tumor size is a prognostic factor for MEN1 related PanNETs. In addition, an expert panel decided whether liver metastases were most likely PanNET related or non-PanNET related. Although we studied a relative large population, statistical analyses were hampered by the small absolute number of patients with WHO G2 tumors and by the small absolute number of patients with liver metastases. Moreover, it should be mentioned that development of liver metastases was the main outcome measure of this study. As the cause of death was not related to metastatic PanNET in a relatively high number of patients and only two patients died of metastatic disease, survival was not considered to be a representative outcome measure for this study. Liver metastases or other distant metastases have been shown to be associated with a higher risk of death in MEN1 patients with PanNETs [5,17,24]. In our study, we did not focus on other distant metastases apart from liver metastases. Only one patient in our cohort had bone metastases from a pancreatic NET and the diagnosis was simultaneous with the liver metastases diagnosis. Lung metastases were not taken into account, as it might be impossible to differentiate lung metastases from primary lung NETs.

The prognostic value of WHO grade in sporadic PanNETs has been shown in various studies [8,20,25] but until now no data were available on MEN1 related PanNETs. Our data show that determining WHO grade can be of prognostic value in non-functioning PanNETs of >2 cm. Patients with WHO G2 tumors according to mitotic count seem to be at risk for development of liver metastases. Although our data are limited, it can be argued that these patients should carefully be monitored. On the other hand, patients with large, WHO G1 tumors are not immune to the development of metastases and follow up is also recommended for these patients. We could not find a significant association between WHO grade according to the standard definition (highest of both Ki67 LI and mitotic count) and the development of liver metastases.

The current WHO classification for neuroendocrine tumors of the digestive tract requires both Ki67 LI and mitotic count for grading and the highest of the two determines the grade [14]. In our
study we had eight (12%) discrepancies between WHO grade based on mitotic count and WHO grade based on Ki67 LI. In three of these cases, WHO grade based on mitotic count was the highest. It is known that discrepancies between mitotic count and Ki67 LI exist in sporadic PanNETs [16,21]. We have no explanation for the discrepancies that we have seen in our cohort. The best method for determining Ki67 LI is a matter of debate, especially regarding values in grey-areas (Ki67 LI 2–3%), and digital image analysis might be more accurate than counting by eye through the microscope [26,27]. In our study, the association between Ki67 LI and liver metastases did not improve when digital image analysis was used. Therefore, we were not able to show prognostic value of Ki67 LI in MEN1 PanNETs, which might be explained by the limitations of this study as discussed before.

Tumor size is a generally accepted prognostic factor for PanNETs in MEN1 but support for this notion from the published literature is scarce. Current MEN1 guidelines recommend to consider surgery for PanNETs >1 cm [7], but it has also been proposed to perform surgery for PanNETs >2 cm [17]. Triponez et al. showed that tumor size of non-functioning PanNETs was associated with lymph node and distant metastases [17]. Tumor size >3 cm was associated with a reduction in survival time. Another study focusing on PanNETs of all types found a correlation between tumor size and distant metastasis. In that study none of the 19 PanNET <2.5 cm had synchronous distant metastases compared to 5 of 22 PanNETs >2.5 cm [5]. The results of our study are consistent with these results. In our cohort of MEN1 patients that underwent surgery for PanNET, liver metastases did not occur in patients with PanNETs <3 cm. An association between tumor size and liver metastases, aggressive growth and survival has also been found in MEN1 patients with Zollinger-Ellison syndrome [24,28,29]. Tumors >3 cm were associated with poor prognosis. These results should be interpreted with caution. It is not known whether the PanNETs included in these studies where gastrinomas or non-functioning PanNETs with co-occurring gastrinomas in de duodenum. The latter option seems to be more likely in MEN1 [6].

In summary, WHO grade based on mitotic count seems to be of prognostic value in non-functioning PanNETs larger than 2 cm in patients with MEN1 and should be included in pathology reports. Patients with WHO G2 PanNETs should carefully be monitored for the development of liver metastases. However, patients with resected large WHO G1 PanNETs are at risk for the development of liver metastases. In contrast, for small PanNETs, less stringent follow up strategies are justifiable. Prospective studies are required for evaluation of the prognostic value of WHO grade in MEN1 clinical practices. Future research should focus on the development of new prognostic markers in MEN1 patients with PanNETs specifically.

Disclosure summary

The authors have nothing to disclose.

Funding

This work was supported by an unrestricted grant from Ipsen Pharmaceutical.

References


