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

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ABSTRACT

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.

Introduction

Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant heritable tumor syndrome. The disease is caused by

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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 histo- 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 supervised 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, VIPomoas 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 Pancreaticoduodenectomy (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 experience 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 (GMR) 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].