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ORIGINAL ARTICLE

Non-functioning neuroendocrine pancreatic tumors transforming to malignant insulinomas - four cases and review of the literature

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Abstract

OBJECTIVE: Neuroendocrine tumors of the pancreas (Pan-NETs) are rare, but among the most common neuroendocrine neoplasias. They are mostly slowly growing with a capacity to metastasize, but transition to a higher grade occurs, which lead to a more aggressive tumor phenotype. Very seldom, non-functional tumors can become hormonally active. Here we present four patients with originally non-functional Pan-NETs that subsequently started to produce insulin or its precursors, causing severe hypoglycemia.

METHODS: We reviewed the medical files, biochemistry and radiological investigations. Pathology tissue samples were re-examined, and additional immunohistochemical analyses were performed.

RESULTS: Four patients; three women and one man, aged 51, 61, 65 and 68 years at diagnosis developed malignant insulinomas 2, 5, 6 and 7 years respectively after initial diagnosis of non-secreting Pan-NETs. They had all metastatic disease at diagnosis. Ki-67 was initially 2, 5 and 6% and progressed to 25, 17 and 45%, respectively. In one patient the initial Ki-67 was 5% but was not reexamined. All four patients died due to their cancer disease within 12, 6, 19 and 29 months after treatment for hypoglycemia commenced. The clinical profile and/or review of the histopathology confirmed all original lesions as non-functional Pan-NETs with subsequent transformation into insulin-producing tumors.

CONCLUSIONS: Non-functional, metastatic Pan-NETs may transform to insulin secreting lesions, with negative impact on prognosis. Therefore, if symptoms as hypoglycemia develops continuous follow-up of clinical parameters, biochemical profiles of pancreatic hormones and histopathological evaluation of proliferation is suggested to detect changes in characteristics of these malignant neoplasms.

INTRODUCTION

The incidence of neuroendocrine pancreatic tumors (Pan-NETs) is 0.5-1/100 000. Of these, 40% secrete hormones, and 60% are non-functional. When non-functional lesions are symptomatic, the most common presenting symptoms are abdominal pain (35–78%), anorexia and nausea (45%) as well as weight loss (20–35%) (Matthews *et al.* 2000). Symptoms can in functional tumors be derived by many variants of hormone producing Pan-NETs, and the clinical picture depends on which hormone is secreted (Falconi *et al.* 2016).

Pan-NETs can also be incidental findings when radiology is performed for other reasons, pancreatic incidentalomas. Most functioning and non-functioning Pan-NETs occur sporadically, but they can also be diagnosed in the work-up of patients with hereditary forms as multiple endocrine neoplasia type 1 or von Hippel Lindau disease. Neuroendocrine lesions in the pancreas are often slowly growing with a potential to metastasize (Halfdanarson *et al.* 2008). Surgery should be performed if tumors are hormonally active and if a tumor is larger than 2 cm. In non-secreting tumors of 1-2 cm in size, there are controversies whether to operate or not (Cheema *et al.* 2012). The best prognostic factor for progression is the Ki-67 index (Boninsegna *et al.* 2012). ^{68}Ga-DOTA-TOC-PET/CT is the imaging technique with highest sensitivity to localize Pan-NETs, however, the sensitivity for detecting insulinomas has been estimated to 25% (Sharma *et al.* 2016). This in turn has been questioned, as in small series, 11 out of 13 and 9 out of 10 insulinomas respectively were found by ^{68}Ga-DOTA-TOC-PET/CT (Prasad *et al.* 2016; Nockel *et al.* 2017).

Insulinomas are Pan-NETs secreting insulin. Different diagnostic criteria are used to establish endogenous hyperinsulinemia. Mainly, elevated insulin levels or symptoms as palpitations, tremor, confusion, personality changes or seizures secondary to hypoglycemia or both, with a plasma concentration of glucose <55 mg/dl (3.0 mmol/l), insulin ≥3.0 μU/ml (18 pmol/l), C-peptide ≥0.6 ng/ml (0.2 nmol/l) and/or proinsulin ≥5.0 pmol/l are proposed (Cryer *et al.* 2009). In contrast to other Pan-NETs, imaging with endoscopic ultrasound detects most of insulinomas (Kann 2018), but whole-pancreas CT perfusion for suspected insulinomas may be superior (Zhu *et al.* 2017). In cases where the lesions are difficult to localize, an arterial calcium stimulation into the main pancreatic arteries and simultaneous venous sampling for insulin (ASVS) detects most remaining insulinomas (Morganstein *et al.* 2009). Of all insulinomas, 10% are considered malignant and exhibit local invasion or distal metastasis. Cure can in the majority be achieved after surgical enucleation. In malignant insulinomas, and in some cases as pre-operative treatment, hypoglycemia is controlled by frequent small meals, glucose infusion, glucocorticoids, the K_{ATP} channel activator diazoxide, somatostatin analogues or the mTOR inhibitor everolimus. In malignant disease peptide receptor-targeted radiotherapy (PRRT) or transarterial chemoembolization (TACE) are additive treatments to avoid hypoglycemia (van Schaik *et al.* 2011; Ito *et al.* 2012).

Here we describe four patients with non-functioning Pan-NETS, which in time transformed to aggressive insulin secreting lesions. The tumors in these patients displayed both a gradual increase in proliferation and clinical symptoms, the latter due to the advent of the tumor's synthesis and secretion of insulin or its precursors. All patients developed malignant insulinomas with recurrent episodes of hypoglycemia resistant to medication. This has been described in very rare cases (Sugiyama *et al.* 2010; Vashi *et al.* 2011; Yoshioka *et al.* 2015; Crona *et al.* 2016).

The aim of this publication is to present the clinical picture, histopathology transformation, discuss causation and treatment possibilities.

MATERIALS AND METHODS

Medical files were reviewed manually in three of the patients recently treated by us for their non-functioning Pan-NETs that transformed to malignant insulinomas (Case 1-3). We registered radiology, laboratory work up and initial and repeated pathology, including an autopsy performed by CCJ. All immunohistochemical staining employed in the re-examination process was performed using clinically approved methodology using a routine pathology laboratory setting and an automated Ventana Benchmark Ultra system (Ventana Medical Systems, Tucson, USA) at our department. The Ki-67 index was calculated by counting the percentage of positive tumor nuclei in 2000 cells in hot spot areas. Further, files of all Pan-NETs treated by us 2006 - 2017 with ICD codes C 25.4 (malignant neoplasm of pancreas, islet of Langerhans), C 25.9D (malignant neoplasm of pancreas, unspecified) and D 13.7 (benign neoplasm of pancreas) were re-examined whereby we found one additional case progressing from non-functional Pan-NET to a malignant insulinoma (case 4).

The study was approved by the local ethical review board in Stockholm 2018/1909-31, and informed consent to review medical files and publish was obtained from relatives to three of the deceased patients, and directly and in writing from the fourth while still alive.

RESULTS

By re-examination of patient charts and ICD codes, a total of 131 Pan-NETs were found, of these 28 (21%) were secreting hormones initially and whereof 16 were insulinomas. Our four patients were initially diagnosed with biologically non-functional Pan-NETs. All had metastatic disease at diagnosis. These tumors progressed after median 5.5 years (2-8). During this time Ki-67 also increased in three patients; from initially 2,

Tab. 1. Biochemical analyses in Case 1-4 at diagnosis and after follow-up

	Case 1 at diagnosis	Case 1 after 8 years	Case 2 at diagnosis	Case 2 after 6 years	Case 3 at diagnosis	Case 3 after 7 years	Case 4 at diagnosis	Case 4 after 2 years	References
fS-c-peptide	1.0	1.2	0.86	1.1	0.40	1.2	1,5	1,8	0.25-1.0 nmol/L
S-Pro-insulin	9.4	190	4.5	289	8.8	516	94	106	3.3-28 pmol/L
fS-Insulin	NA	13	73.2	18.6* (<11mE/L)	3.3	25**	147***	571****	2.0-25 mIE/L
P-glucose	8.3	3.8	6.6	1.8	10.5	2.7**	5,5	2,1	4-6 mmol/L
HbA1c	89	53	NA	41	5.8%°	57	NA	NA	31-46 mmol/ mol
fP-Chromo-granin A	5.5	12	7.2	6.4* (<3 nmol/L)	291	542	55	80	< 3.0 nmol/L
fP-Glucagon	60	32*	120	8.6* (<18pmol/L)	219°°	74	69	62	< 60 mmol/L

*Different biochemical tests in 2016; ** 2018, 8 years after diagnosis, NA; not available; *** reference 18-73 pmol/L; **** reference < 140 pmol/L; ° reference 4-6%; °° reference 0-250 mmol/L

6 and 5% (median 5), respectively to 25, 17 and 45% (median 25). At late stage hypoglycemic symptoms became apparent. All four patients deceased 6, 12, 19 and 29 months (median 15.5) after they needed treatment for hypoglycemia, which by time became impossible to treat, although two were in reasonably good clinical shape. Details in each case history are given below and in Table 1. Major gross and histological findings of each tumor from three patients are depicted in Figure 1-3 and additionally listed in Table 2.

Case 1

In 2013, a 68-year old woman with asthma, hypothyroidism, hypertension and type two diabetes treated with metformin developed abdominal pain. She was subsequently diagnosed with a thrombus in the portal vein secondary to a 6-8 cm large pancreatic tumor, which was judged to be inoperable, also growing *per continuum* into the liver. A fine-needle aspiration biopsy (FNAC) with endoscopic ultrasound verified the lesion as a Pan-NET that expressed synaptophysin

Tab. 2. Chronological scheme regarding histopathological characteristics of the three cases

Case no.	Year	Material	Diagnosis	Ki-67 index (%)	WHO grade	CHGA	SYP	PDX1	ISLET1	INSM1	Insu-lin	Gluca-gon	SOM	PP
1	2013	FNAC of pancreatic tumor	Pan-NET	2*	1	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1	2018	Core biopsy of liver metastasis	mPan-NET	6	2	+	+	+/- (30%)	+	+	-	-	+/- (10%)	+/- (2%)
2	2010	Core biopsy of liver metastasis	mPan-NET	6	2	+/- (75%)	+	+/- (10%)	+	+	-	-	-	-
2	2012	Core biopsy of liver metastasis	mPan-NET	7	2	+	+	+	+	+	-	-	-	-
2	2014	Core biopsy of liver metastasis	mPan-NET	16	2	+	+	+	+	+	-	-	-	-
2	2016	Core biopsy of liver metastasis	mPan-NET	17	2	+/- (40%)	+	n.d.	n.d.	n.d.	+/- (20%)	-	-	+
3	2010	Partial pancreatectomy	Pan-NET	5	2	+	+	-	n.d.	+	+/- (5%)	-	-	-
3	2016	Partial liver resection	mPan-NET	45	3	+	+	+/- (40%)	+	+	+/- (30%)	-	-	-

FNA; fine needle aspiration biopsy, Pan-NET; Pancreatic neuroendocrine tumor, mPan-NET; metastatic pancreatic neuroendocrine tumor, * Ki-67 index based on immunocytochemistry; +; positive immunoreactivity, +/-; mixed immunoreactivity, -; negative immunoreactivity; nd; not determined; (X %); proportion of positive cells for each hormone with mixed immunoreactivity; CHGA; chromogranin A, SYP; synaptophysin, SOM; somatostatin, PP; pancreatic polypeptide.

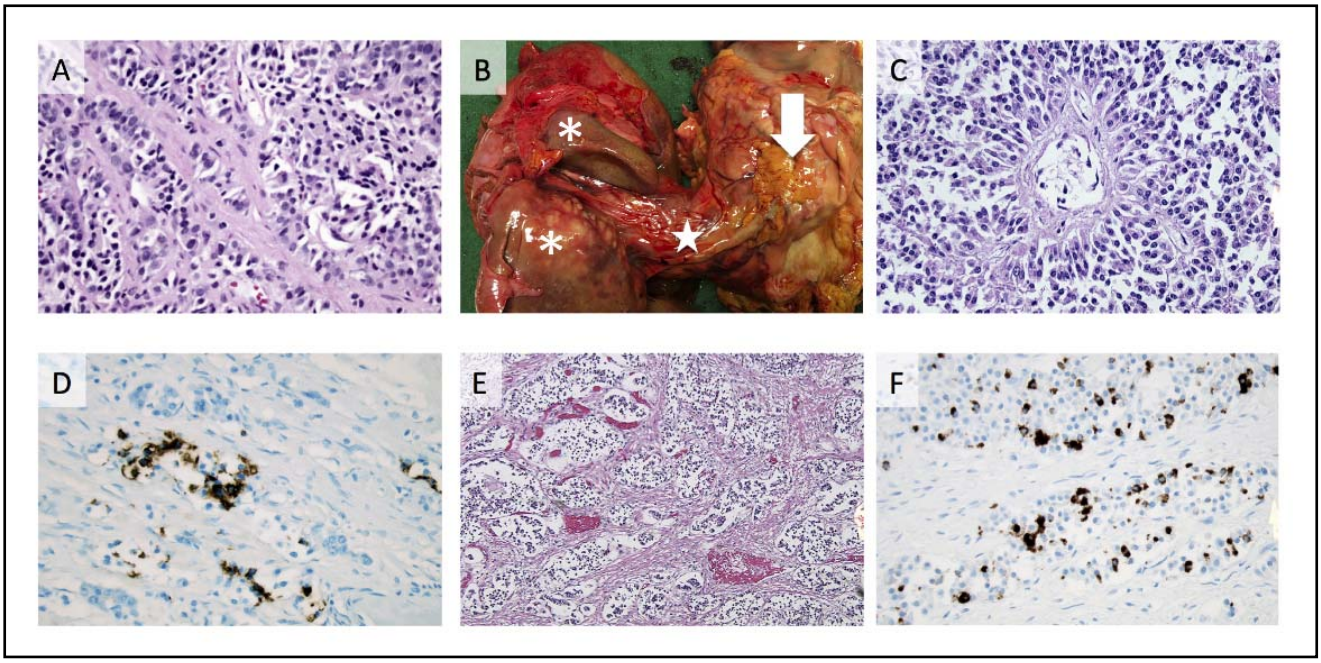

Fig. 1. Histological and autopsy findings from case 1 at different time points. A) Core needle biopsy from the liver depicting H&E (haematoxylin&eosin) staining of a metastatic Pan-NET WHO grade 2 without demonstrable insulin production. This lesion was positive for neuroendocrine markers and displayed a Ki-67 index of 6% (data not shown). **B)** Gross autopsy findings of the 21 cm pancreatic mass (arrow) extending directly into the liver (asterisk) through the hepatoduodenal ligament (star). **C)** H&E staining from the pancreatic mass, revealing a Pan-NET WHO grade 3 (x400 magnification). **D)** Focal insulin production in approximately 1% of the primary Pan-NET cells. **E)** H&E staining from the ovarian mass, depicting metastatic Pan-NET WHO grade 3 (x100 magnification). This lesion was positive for Chromogranin A and synaptophysin (data not shown). **F)** Insulin immunoreactivity in the ovarian metastasis at x400 magnification, displaying intense immunoreactivity in approximately 30% of tumor cells.

and chromogranin A. The cytological Ki-67 index was appreciated to 2% (Table 2). Low-molecular heparin and somatostatin analogs were administered. There were initially no other known metastases.

One year later, the patient exhibited hyperglycemia and was treated with insulin. Three years after this onset, at a time point when the patient was asymptomatic, there was radiological evidence of a solitary metastasis in the pelvis. In early 2018, the patient was found to be hypoglycemic, and insulin was withheld. Investigation with ^{68}Ga-DOTA-TOC-PET/CT revealed tumor progression during the last three years, from a tumoral size of 9x5 cm in the liver and 8x5 cm in the pancreas, to 13x8 and 10x6 cm respectively. She gradually developed more severe hypoglycemic symptoms. By this time, the pelvic metastasis had also grown. A core needle biopsy of the liver was performed and was consistent with a metastatic Pan-NET WHO grade 2. Immunohistochemical analyses showed a Ki-67 proliferation count at 6%, as well as focal immunoreactivity towards somatostatin and pancreatic polypeptide (PP) (Figure 1, Table 2). No immunoreactivity was observed for insulin, but the large tumor burden plus the observed hypoglycemia still argued for a focal insulin production clinically elsewhere in the primary tumor, or in areas of the metastatic lesion that were not investigated through the biopsy. She started treatment with everolimus 2.5 mg x 1, octreotide 50 ug x 2 and prednisolone 30 mg x 1, with initially a good response in blood glucose, but she later become nonresponsive to treatment. During late palliative care, treated with only everolimus, she passed away.

An autopsy was performed, and the cause of death was bilateral pneumonia and concomitant pulmonary embolism with occlusion of the truncus pulmonalis. In addition, a 21 cm large pancreatic tumor was observed, grossly infiltrating both liver lobes by direct extension (Figure 1). The tumor mass also invaded the peripancreatic fat and spleen. Furthermore, a 7 cm mass in her right ovary was also seen. Microscopy of the pancreatic lesion revealed a Pan-NET WHO grade 3 (Pan-NEC) and a distinct immunoreactivity to insulin in approximately 1% of the tumor cells, and with multiple metastases to the liver and spleen (Figure 1). Ki-67 was calculated to be 45%, with the notion that this analysis could be potentially affected by the post mortal state. The ovarian mass was found to be metastatic Pan-NEC with immunoreactivity towards insulin in approximately 30% of the tumor cells (Figure 1).

Case 2

A 61-year old man with a history of ischemic heart disease, atrial fibrillation and hypertension presented with epigastric and lumbar pain associated with increased bladder activity in 2010. A computed tomographic (CT) scan of the abdomen revealed a tumor in the pancreatic body and tail, and multiple metastases in the liver. A core needle biopsy of the liver showed infiltrating

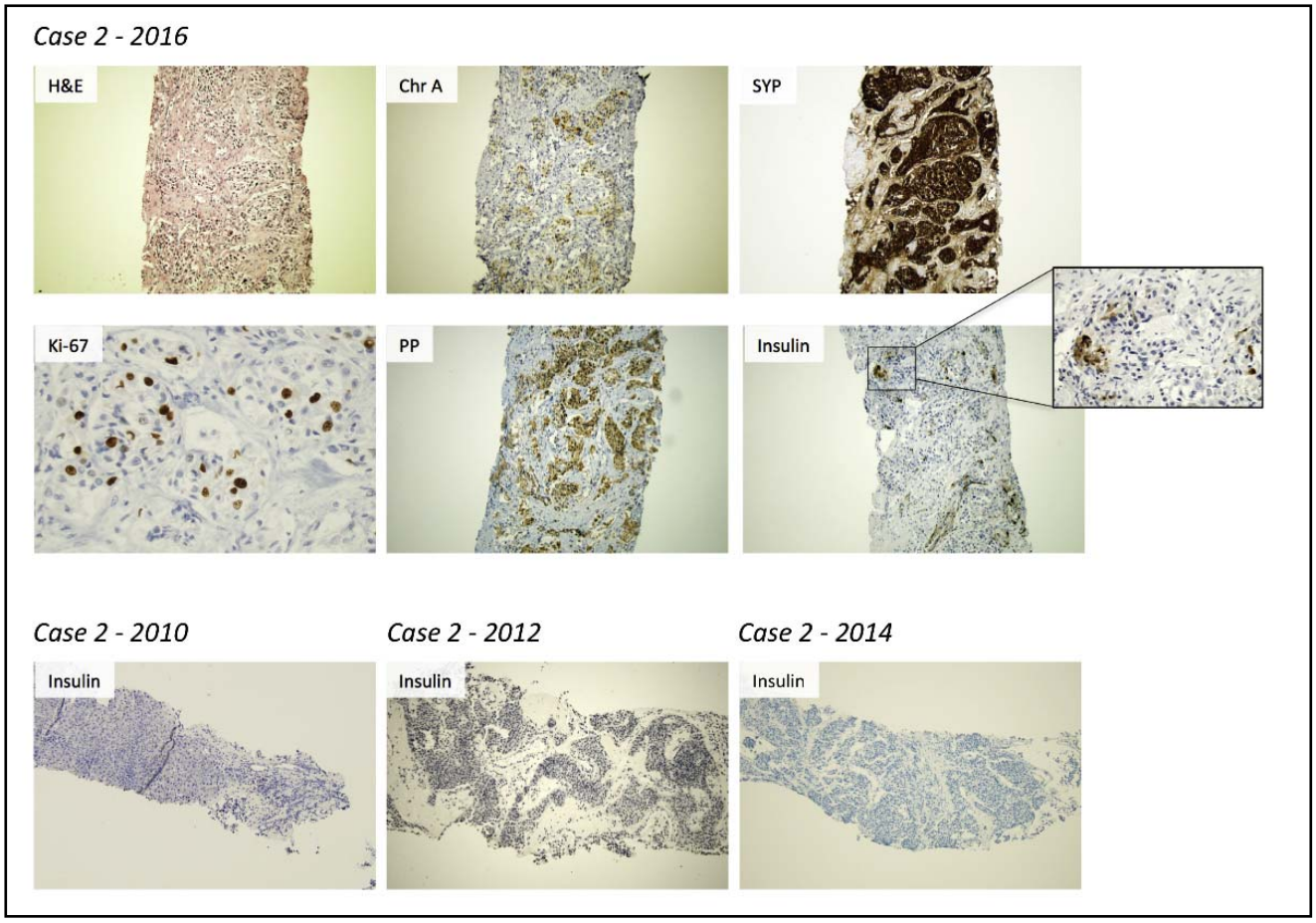

Fig. 2. Photomicrographs of core needle biopsy material from case 2 at different time points. H&E (haematoxylin&eosin) staining depicts an infiltrating tumor with trabecular growth, which stained positive for Chr A (chromogranin A) and SYP (synaptophysin), thereby confirming the metastasis as a neuroendocrine tumor. The Ki-67 index was 17%, thereby reaching WHO grade 2. The tumor was also positive for PP (pancreatic polypeptide) and focally for insulin. The bottom row depicts three previous core needle biopsies (2010, 2012, 2014) with negative immunoreactivity for insulin. All images are magnified x100 except for the Ki-67 photomicrograph and the 2016 insulin stained insert that were both magnified x400.

tumor cells in trabecular formations. One mitosis per 10 HPF was observed, but no tumor necrosis. Moreover, immunoreactivity for synaptophysin, chromogranin A, ISLET1, INSM1 as well as focal staining for PDX1 was seen, and a Ki-67 proliferation index of 6 % was observed. No immunoreactivity was seen for pancreatic hormones (Figure 2, Table 2). The findings were congruent with a metastatic Pan-NET WHO grade 2 without evident hormone production. The patient had elevated plasma levels of pancreastatin, gastrin and pancreatic polypeptide without a clinical correlation (pancreastatin 29 pmol/L (<15), gastrin 511 pmol/L (<50), pancreatic polypeptide 499 pmol/L (<100), Table 1). Further radiological investigations demonstrated high expression of somatostatin receptors and treatment with a somatostatin analog was initiated. The patient also received six cycles of chemotherapy with Cisplatin/Etoposide during 2011. He developed neuropathy in his lower extremities, but no progress in disease was detected after the first three cycles.

In February of 2012, CT and ^{68}Ga-DOTA-TOC-PET/CT investigations observed progression of the liver metastasis and lymph nodes in the abdomen. Furthermore, an additional core needle liver biopsy showed a Pan-NET with similar immunohistochemical profile as the previous biopsy two years earlier, including a proliferation rate of 7 %. At this point, laboratory tests demonstrated increased gastrin and glucagon levels (76 and 210 pmol/L respectively), but normal calcitonin, insulin and proinsulin levels. PRRT treatment was initiated as a second line treatment. After a third cycle of PRRT, radiology showed a reduction in tumor size, both in the pancreas and liver. The patient received in total five cycles of PRRT.

After completed treatment, further tumor progression was detected in 2014, in which a core needle biopsy of the liver confirmed the tumor as WHO grade 2 (Ki-67 16%), still without immunoreactivity to antibodies targeting pancreatic hormones. The therapy was changed to Capecitabine/Temozolomide and later to 5 mg everolimus, which were both discontinued due to bone marrow suppression. Slow tumoral progression was observed, and a TACE embolization was performed in 2016.

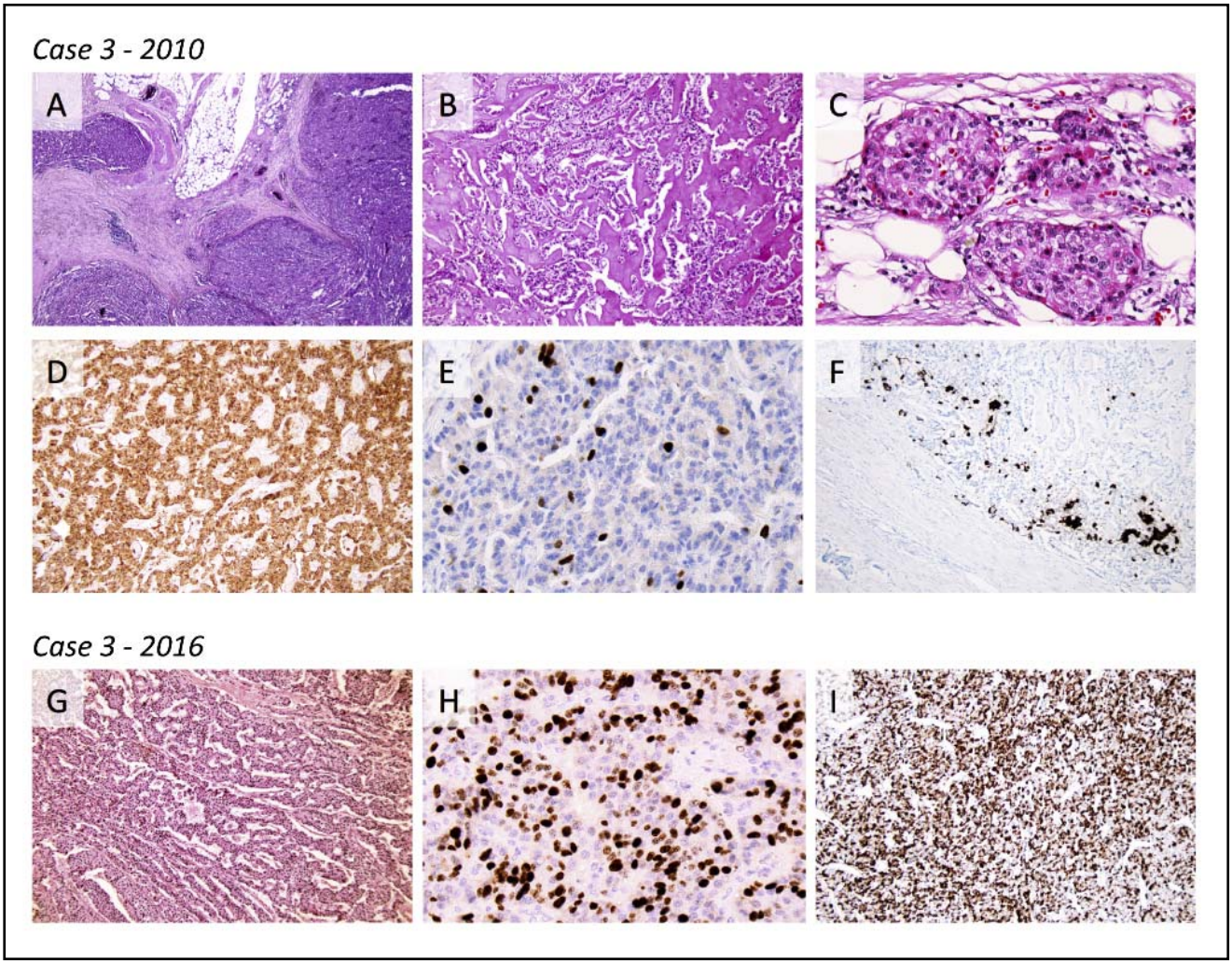

Fig. 3. Photomicrographs from case 3 and the partial pancreatectomy in 2010 and partial liver resection in 2016 respectively. A) H&E (haematoxylin&eosin) staining at x40 magnification displaying tumoral infiltration of peripancreatic adipose tissue. **B)** H&E staining at x100 magnification highlighting the hyaline stroma surrounding the tumor. **C)** H&E staining at x400 depicting foci with angioinvasion. **D)** The tumor cells were positive for chromogranin A, verifying the tumor as neuroendocrine (x100 magnification). **E)** Ki-67 proliferation index was 5%, thereby establishing the lesion as a WHO grade 2 tumor (x400 magnification). **F)** Focal insulin immunoreactivity in 5% of the tumor cells was noted (x100 magnification). **G)** H&E staining at x100 depicting the Pan-NET metastasis with a predominant well-differentiated growth pattern. **H)** Ki-67 immunoreactivity at x400 magnification, displaying 45% positive cells, verifying the tumor as a Pan-NET WHO grade 3 according to the 2017 WHO criteria. **I)** Diffuse and widespread insulin immunoreactivity at x100 magnification.

Later that year, the patient reported the first symptoms of hypoglycemia, with attacks of sweating, tremor and nausea a few times a week. Blood glucose was during such an attack 2.8 mmol/L. A new liver biopsy now showed an increased Ki-67 index of 17%, and by now the tumor tissue was found focally positive for insulin (Figure 2, Table 2). In the autumn of 2016, radiology investigations demonstrated further progress in tumor mass and presence of skeletal metastases in the vertebral column. A new set of Lutetium therapy was initiated, this time four treatments were given. In August of 2017, low blood glucose was again detected, and the patient now reported that he experienced hypoglycemic attacks with tremor in fingers and prickling in the tongue several times a day even though he had more frequent meals. Prolonged supervised fasting test was interrupted after four hours because of hypoglycemic symptoms and blood glucose of 1.8 mmol/L. Everolimus treatment was reintroduced and continuous intravenous glucose infusions during inpatient care were required. Due to respiratory symptoms, assessed as a suspected adverse effect of everolimus, the medication was again discontinued. Pasireotide 0.6 mg iv twice daily had no effect on blood glucose, and a trans-arterial chemoembolization (TACE) had moderate effect on glucose levels. Diazoxide 100 mg twice a day also exhibited modest effects, and due to liver and kidney failure the medication was discontinued after a few days of treatment. The patient died due to multi-organ failure.

Case 3

This 65-year old female patient had an earlier medical history of a biliary cyst of the left hepatic lobe. During

follow up of the cystic lesion, a suspected neuroendocrine tumor was found in the pancreas, with metastatic deposits in the liver. In 2010, the patient underwent a left hemipancreatectomy and a concomitant wedge resection of the liver. Pathology confirmed the diagnosis of a Pan-NET WHO grade 2 (Ki-67 index of 5%) with focal immunoreactivity towards insulin (5% of tumor cells) (Figure 3). Post-operatively the patient was diagnosed with diabetes mellitus. ^{68}Ga-DOTA-TOC-PET/CT demonstrated multiple liver and bone metastases, and somatostatin analog treatment was initiated. Four years later, in 2014, the patient received PRRT treatment, in total four cycles. One year later, the patient developed symptoms of hypoglycemia, and symptoms persisted even though insulin treatment for her diabetes was interrupted. An MRI showed progression of a now 6 cm large liver lesion, which was resected with a left lobectomy. The pathology report from of this resected lesion displayed a metastatic Pan-NET WHO grade 3 with a Ki-67 of 50%, staining positive for insulin in 30 % of tumor cells. The tumor cells were positive for chromogranin A, synaptophysin, ISLET1, INSM1 and focally for PDX1 (Figure 3, Table 2). A P53 staining was only partially positive, indicating no underlying *TP53* gene mutation. As the current WHO guidelines from 2017 dictate that well-differentiated lesions with Ki-67 counts between 20-55% and negative/mixed P53 and retained ISLET1 immunoreactivity could be appreciated as Pan-NETs WHO grade 3 instead of Pan-NECs, the former nomenclature was chosen.

Postoperatively there was hyperglycemia and insulin had to be re-introduced. She did not experience any episodes of hypoglycemia but had ongoing insulin treatment. In 2016, there was disease progression with metastasis in the vertebral column and additional liver lesions. Treatment with everolimus 5 mg once daily was initiated, and afterwards a trans-arterial radioembolization (TARE) of the liver metastasis was performed. At this point, she suffered from symptoms of fatigue and loss of appetite. In the end of 2017, she experienced a relapse with severe hypoglycemic symptoms, most frequent during nighttime. Insulin, everolimus and somatostatin analog treatments were discontinued. In the beginning of 2018, treatment with continuous intravenous glucose infusions and octreotide 100 ug x 2 subcutaneously was initiated, which was unsuccessful. This was followed by diazoxide 100 mg x 2 and prednisolone 10+20 mg. Despite these treatments, further progress was observed and in February 2018, a decision was made with the patient. All treatments and glucose infusions were discontinued, and she passed away within hours.

Case 4

A 51-year old female was investigated due to macrohematuria. A CT scan showed liver metastases, and further investigation revealed a 3 cm primary tumor in the tail of the pancreas. A liver biopsy displayed metastatic neuroendocrine tumor with a Ki-67 index of 5%. Immunohistochemistry in sparse remaining tumor tissue was positive for PDX1, ISLET1, INSM1, synaptophysin and CD56, but negative for insulin. The patient started somatostatin analog treatment (lanreotide 120 mg every fourth week), but progressed. Initially, the patient had elevated plasma levels of pancreastatin, glucagon, pro-insulin and c-peptide without a clinical correlation (pancreastatin140 (<15 pmol/L) glucagon 69 pmol/L (<60), pro-insulin 94 pmol/L (3.3-28) and c-peptide 1.5 pmol/L (0.25-1.0)) (Table 2). Insulin and blood glucose were normal, 147 pmol/L (18-173) and 5.5 mmol/L, respectively. As her tumor progressed, treatment with streptozotocin/5-fluorouracil resulted in a partial response after four cycles, with a concomitant decrease in peptide levels. After 8 cycles, the tumor burden progressed. Pasireotide was then commenced together with everolimus by which she experienced weight loss and elevated blood sugar levels. Thereafter, therapy was changed to temozolamide 270 mg once daily for five days, but the therapy was discontinued after two cycles due to severe nausea as well as progress of the disease, and all anti-tumor therapy was withheld. Glucose gradually increased in time and insulin treatment was commenced for two months. Later, insulin had to be withdrawn as her glucose levels were low. Two years after the initial diagnose, her hypoglycemia became difficult to treat. Glucocorticoids with oral betamethasone 0.5 mg, 6+6 was used up to stabilize the patient, until she passed away. No autopsy was performed.

DISCUSSION

Here we describe detailed clinical, biochemical and repeated histopathological data in three patients with metastatic non-functional Pan-NETs, which in time transitioned to a higher grade and to malignant insulin-secreting tumors. This was confirmed by new histopathology and the tumor tissues were positive for insulin in all three cases. Histologic slides for reevaluation was not available for case 4.

One of the patients had diabetes prior to initial diagnosis and two additional patients developed diabetes, one secondary to pancreatic surgery and one possibly drug-related (pasireotide and everolimus), but were forced to gradually stop all diabetic treatments, and instead struggle to avoid hypoglycemia. Such changes in the pattern of a Pan-NETs occur rarely and have previously been published as case reports (Sugiyama *et al.* 2010; Vashi *et al.* 2011). In two retrospective analysis of 323 vs. 435 patients with Pan-NETs, transformation to hormone producing lesions were seen in 14 (4%) and 15 (3.4%) respectively, whereof 50-33% were transformed to insulinomas (de Mestier *et al.* 2015; Crona *et al.* 2016). This phenomenon occurred only in patients with advanced disease, related to biochemical and/or radiological tumor progression as well as impaired

prognosis. In the publications by Crona et. al. and de Mestier *et al.* the median time to transformation was 53 months (range 12-213) and 55 months (range 7-219) respectively, which is in line with 5.5 years as the median time among our patients. The authors also found that prognosis was worse in previously non-functional Pan-NETs with subsequent insulin secretion compared to cases with hypersecretion of other peptides such as gastrin, ACTH, glucagon or calcitonin, although very few such patients were described in those cohorts (de Mestier *et al.* 2015; Crona *et al.* 2016). The median survival time was 10 months in the patients developing insulinoma, compared to our patients that lived 15.5 months from debut of hypoglycemia (Crona *et al.* 2016). This overall implies a worse prognosis in patients with tumors changing from non-functional Pan-NETs to malignant insulinomas, which often refers to cases exhibiting local invasion or distal metastasis.

Some Pan-NETs have also been reported to secrete multiple hormones, as in the cohort reported by Crona et al, 4 out of 14 secreted two pancreatic hormones (Crona *et al.* 2016). In our study, all cases had initially elevated plasma levels of glucagon, three had slightly elevated c-peptide levels, two displayed elevation of pro-insulin and two cases demonstrated increased secretion of insulin. At that time, our patients did not display any hormonal symptoms, and glucagon levels normalized with time. In retrospect, this could warrant follow-up in an extended material, although no hormonal symptoms to these biochemical findings were evident, and indeed three out of four patients had elevated blood glucose necessitating insulin treatment during the course. Interestingly, analysis of pancreatic hormones has not been advocated in non-functional Pan-NETs in the absence of symptoms (Vinik *et al.* 2010).

In the three patients with full information available, the Pan-NETs initially displayed a Ki-67 index of 2 to 6%, which later increased. Crona *et al.* described seven insulinomas, but a potential shift in the Ki-67 index was not investigated in these patients (Crona *et al.* 2016), whereas an increase from 7% to 17% was described by de Mestier et.al., including all tumors with hormonal hypersecretion (de Mestier *et al.* 2015). More commonly, tumor progression can be seen in Pan-NETs without the development of hormonal hypersecretion. In Pan-NET metastases, the Ki-67 index has been reported to be elevated compared to the primary tissue sample in a third of patients (Zen & Heaton 2013; Miller *et al.* 2014). Furthermore, transition to higher tumor grade is rarely described in lesions secreting hormones, in contrast to in our patients with aggressive tumors. The underlying causes of such a process are not well established. Healthy mature cells and tumor cells alike have a capacity to reverse to pluripotent cells, and the de-differentiation could also be driven by a clonal expansion of cells, either present at initial diagnosis or developing subsequently from an increase in damaging DNA events (Gurdon 1962; Takahashi & Yamanaka 2006). In Pan-NETs, somatic mutations in *DAXX, ATRX, PTEN* and *TSC2* have been demonstrated, and *DAXX/ATRX* mutation positive vs. negative Pan-NETs also display different methylation profiles (Jiao *et al.* 2011; Pipinikas *et al.* 2015). If such genetic or epigenetic alterations could explain changes in the hormonal secretion pattern is not known and must be investigated in future studies.

The inherent limitation of retrospective studies, above all that of ascertainment bias, was present in this study as well. Moreover, not all biochemical tests, which in hindsight would have been of value, were analyzed. We can also not accurately assess the association between an increase in Ki-67 index and the development of malignant insulinomas.

We conclude that non-functional neuroendocrine metastatic pancreatic tumors may with time transform to insulin secreting lesions. If hypoglycemia develops, continuous follow-up - not only of clinical, biochemical and radiological parameters but also histopathological investigations of pancreatic hormones and proliferation index - is necessary to detect and treat these malignant neoplasms.

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