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The epithelial-mesenchymal transition induces aggressivity of mucinous cystic neoplasm of the pancreas with neuroendocrine component: An immunohistochemistry study

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ABSTRACT

Background: Pancreatic mucinous cystic neoplasms (MCN) are rare tumors that are usually diagnosed in females.
Materials and methods: In our department, only four of the 109 consecutive cases of pancreatic tumors (3.67%) were diagnosed as MCNs. In this report, we present the characteristics of these four specific cases which also showed unusual HER-2 positivity and neuroendocrine differentiation.
Results: The four MCNs were diagnosed in patients with ages between 46 and 75 years. Other clinical particularities were the following: one benign case, splenic rupture as result of a giant cystic tumor on the tail of the pancreas directly invading the spleen in the second one, metastases in the accessory spleen in the third one and invasion of the abdominal vessels in the fourth case. In all of these cases, the ovarian-like stroma tested positivity for calretinin, progesterone receptor (PR) and, in cases 2 and 3, for AE1/AE3 keratin. The malignant tumor cells were marked by carcinoembryonic antigen, HER-2, maspin, PR and the neuroendocrine markers synaptophysin, CD56, and neuron-specific enolase.
Conclusions: These cases highlight the unusually aggressive behavior of pancreatic MCN with invasive carcinomas that share mixed exo- and endocrine components and show epithelial-mesenchymal transition.

1. Introduction

Pancreatic cystic tumors were classified in 1978 by Compagno et al. as serous and mucinous cystic neopalsm with latent or overt malignancy (cystadenoma and cystadenocarcinoma respectively) [1–4]. The cyst-forming mucinous tumors resemble cysts lined by a tall mucin-producing epithelium with focal papillary structures [1–3]. Based on the presence or absence of communication with the pancreatic duct, they were re-classified in 1996 by the World Health Organization (WHO) into two categories: intraductal papillary mucinous tumors and mucinous cystic tumors, the term “tumor” being later replaced by “neoplasm” [5]. The intraductal papillary mucinous neoplasms (IPMNs) were then categorized by duct size into main duct, branch duct and peripheral IPMNs [5–7]. To avoid confusion, enlargement of the main duct is required for the diagnosis of main duct IPMN, whereas involvement of the small ducts without dilatation of the main duct is accepted for the diagnosis of branch duct IPMN. Tumors that do not display macroscopic communication with the ducts are categorized as mucinous cystic neoplasms (MCNs) and are almost exclusively located in the pancreatic body and tail [5–7].

The current diagnosis and management of MCNs is based on criteria established at the Consensus Conference of the International Association of Pancreatology which took place in 2004 in Sendai, Japan. The Sendai consensus guidelines were published in 2006 [6] and updated after the 14th meeting of the International Association of Pancreatology in Fukuoka, Japan, in 2010. These updated guidelines were published in 2012 [7]. In 2015 a revised terminology was proposed [8].

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In order to diagnose MCN, the last adapted consensus and WHO criteria [1,8] require lack of communication with the main pancreatic duct as well as the presence of the ovarian-type stroma beneath the epithelium [2,3,5–7,9,11]. The tumor must also be predominantly (75–98%) located in the pancreatic body or tail [2,3,5–7,9–11].

MCN occurs most frequently (in 90–98% of cases) in perimenopausal women and the male to female ratio is 1:20 [1–3,5,8,9]. Microscopic communication with the main pancreatic duct may be occasionally found [5]. Scanty fibrotic or hypocellular stroma can be seen in elderly males and postmenopausal women [6].

Based on the 2010 WHO classification of MCNs and dysplasia grading, these tumors are categorized as neoplasms with low- or intermediate-grade dysplasia/benign tumors (70% of the cases) and with high-grade dysplasia/borderline tumors (20% of MCNs). Malignant cases (6–36% of MCNs) were called “MCNs with an associated invasive carcinoma” [1,5]. The newest consensus that was published in 2015 [8] recommended another revised terminology as follow: MCNs with low- or intermediate-grade dysplasia/benign tumors are now considered MCNs with low-grade dysplasia whereas MCNs with high-grade dysplasia/borderline tumors should be diagnosed as MCNs with high-grade dysplasia/carcinoma in situ. The MCNs with an associated invasive carcinoma should be now reported, based on the percentage of the invasive component, as “MCNs with an associated invasive carcinoma or invasive carcinoma with an associated MCN” [8]. These denominations should be currently used in the daily diagnosis, as they have been included in the 2017 AJCC Cancer Staging manual – 8th edition [12].

The invasive component is usually a tubular or ductal adenocarcinoma, but anaplastic or undifferentiated carcinomas and choriocarcinomas were also described [5,13]. The immunoprofile of the tumor cells is based on the presence of epithelial membrane antigen (EMA) and keratins 7, 8, 18 and 19. Other antibodies such as keratin 20, carcinoembryonic antigen (CEA), DUPAN-2 and CA 19-9 are occasionally expressed [1,5]. The ovarian-type stroma cells usually express progesterone receptors (PR, PR-a and PR-B) and estrogen receptors (Er-α and ER-β) but can also display positivity for androgen receptor, steroidogenic factor (SF-1), calretinin, vimentin, tyrosine hydroxylase, smooth muscle antigen (SMA), α-inhibin, melan-A, CD99, bcl-2 and even human chorionic gonadotropin (hCG) [1,5,6,11].

As MCNs represent only 8% of all cystic lesions of the pancreas [10], there is little data to be found in the literature about their specific features. Only 77 representative papers about MCNs were reported between 1996 and 2009 [5] and about 210 from 2010 to 2016. In 2017 the number of papers increased significantly but most of these reports were centered on the criteria of differentiation between MCN and IPMN or take into account the genetic origins of these tumors.

In this paper, we present four consecutive cases of pancreatic MCNs with various aspects, one MCN, low-grade and three MCNs with associated invasive carcinomas. We also showed for the first time in literature a possible neuroendocrine differentiation of these tumors and proved that the aggressivity of MCNs might be related on the epithelial mesenchymal transition (EMT) of tumor cells.

2. Materials and methods

To select the MCNs, we have retrospectively checked all of the pancreatic lesions diagnosed during 2012–2016 in our Pathology Department. From the 183 consecutive cases, 74 were pancreatitis and 109 were diagnosed as pancreatic tumors.

The Ethical Committee approval was obtained for performing this study. For the four cases presented in detail, signed written consent was obtained from the patients for surgical intervention and publication of the case details.

The immunohistochemical analysis were manually performed using the EnVision Flex Kit (Dako, Glostrup Denmark). The details regarding the used antibodies were inserted in Table 1.

Table 1
The characteristics of the immunohistochemical markers.

IHC marker (company)	Clone	Dilution
Cytokeratin (DAKO)	AE1/AE3	1:50
EMA (DAKO)	E29	1:100
CEA (DAKO)	II-7	1:50
Maspin (Novocastra)	EAW24	1:25
PR (Novocastra)	16	RTU
ER (Novocastra)	6F11	RTU
Chromogranin (DAKO)	DAK-A3	1:100
Synaptophysin (DAKO)	DAK-Synap	1:100
NSE (DAKO)	BBS/NC/VI-H14	1:100
CD56	123C3	1:50
HER-2 (DAKO)	5A2 cerbB2-oncoprotein	1:1000
CD105 (Novocastra)	Monoclonal mouse	1:100
DOG1 (Novocastra)	NCL-L-DOG1	1:50
Calretinin (DAKO)	DAK-Calret 1	1:50
Inhibin α (DAKO)	R1	1:50
p53 (Novocastra)	DO-7	1:50
E-cadherin (DAKO)	NCH-38	1:50
N-cadherin (DAKO)	6G11	1:100
SLUG (Santa Cruz Biotech)	Rabbit Polyclonal	1:100
Vimentin (DAKO)	V9	1:800

(EMA = epithelial membrane antigen; CEA = carcinoembryonic antigen; ER = estrogen receptor; IHC = immunohistochemical; NSE = neuron specific enolase; PR = progesterone receptor).

3. Results

The histopathological subtypes of the 109 pancreatic tumors were the following: 89 ductal adenocarcinomas (81.65%), 13 neuroendocrine carcinomas (11.93%), four cases of MCNs (3.67%), two serous adenomas (1.83%) and one adenosquamous carcinoma (0.92%). In this paper, the detailed presentation of the four MCNs was performed (Table 2).

Case 1. A 46-year-old non-alcoholic/non-smoker previously healthy female (160 cm height and 68 kilos weight) presented with mild epigastric pain and long-time history of gastro-esophageal reflux (heartburn). Besides hypertension (160/110 mm Hg), no significant medical history was revealed.

At palpation, a soft-consistency, painless nodular mass was identified in the left hypochondrium. The CT scan revealed a large, septated cystic neoplasm of the pancreatic tail with gastric compression; the presumptive diagnosis was a hydatid cyst (Fig. 1). Laboratory test results fell within normal ranges (blood amylase - 108 U/L [normal ranges 25–125 U/L]; blood glucose – 113.3 mg/dL [normal ranges 80–120 mg/dL]).

A laparoscopic resection of the pancreatic tumor was performed and the surgical specimen was sent to the Pathology Department. Macroscopically, a 40 × 15 × 8 mm cyst wall with smooth surface was described. Under microscope, the cyst was shown to be lined by tall mucin-producing epithelium with low-grade dysplasia, without an invasive component (Fig. 1). The underlying ovarian-type stroma expressed PR, ER, calretinin and SLUG and tested negative for AE1/AE3 keratin and CD105 (Table 2).

The final diagnosis was “MCN of the pancreatic tail with low-grade dysplasia” according to the WHO updated criteria [8,12]. Postoperatively, both amylasemia and amylasuria levels fell within normal ranges (62 U/L in the blood and 255 U/L in the urine [normal ranges < 460 U/L]). The patient was discharged seven days after surgical intervention with normal serum and urinary levels of amylase and glucose.

Three months after surgery, she was re-hospitalized for chronic cholecystitis and a laparoscopic cholecystectomy was performed. The CT scan revealed a solid area in the remaining pancreatic tail. The patient refused surgical excision. Without any therapy, the patient is still alive at 12 months after surgery without any complaints.

Table 2
Clinicopathological, histological and immunohistochemical characteristics of pancreatic mucinous cystic neoplasm with/without associated invasive carcinoma.

Parameter	Case 1	Case 2	Case 3	Case 4
Gender	Female	Male	Female	Male
Age	46 years	65 years	72 years	57 years
Tumor localization	Pancreatic tail	Pancreatic tail and spleen, with peritoneal carcinomatosis	Pancreatic tail, celiac trunk, spleen, mesenteric and splenic arteries, gastric wall, left adrenal gland and left kidney, with peritoneal carcinomatosis	Pancreatic body, celiac trunk and common hepatic artery
Type of surgery	Laparoscopic cystic resection	Distal pancreatectomy, splenectomy and epiploic nodules resection	Distal pancreatectomy, splenectomy and left adrenalectomy	Corporeo-caudal pancreatectomy and lymphadenectomy
Survival time (after surgery)	Alive at 12 months after resection	8 months	4 months	7 months
Histopathological tumor type	MCN with low grade dysplasia, without invasive component	MCN with invasive ductal adenocarcinoma with neuroendocrine differentiation	MCN with invasive ductal adenocarcinoma with neuroendocrine differentiation	MCN with invasive ductal adenocarcinoma
Tumor stage	Benign tumor	pT3NxM1 (stage IV)	pT3N1M1 (stage IV)	pT3N1M0
Tumor stroma - immunoprofile				
Positive markers	ER, PR, calretinin, SLUG	PR, calretinin, SLUG, AE1/AE3 keratin, vimentin, CD105	PR, SLUG, AE1/AE3 keratin	
Negative markers	AE1/AE3 keratin, CD105	ER	ER, calretinin, vimentin, CD105	AE1/AE3 keratin
Tumor cells - immunoprofile				
Positive markers	AE1/AE3 keratin, EMA, E-cadherin	AE1/AE3 keratin, EMA, E-cadherin, vimentin, maspin, HER-2, chromogranin, synaptophysin, NSE, CD56, CEA, p53	AE1/AE3 keratin, EMA, E-cadherin, vimentin, maspin, HER-2, chromogranin, synaptophysin, NSE, CD56, CEA, p53	AE1/AE3 keratin, EMA, E-cadherin, vimentin, maspin, HER-2, CEA, p53
Negative markers	CEA, Maspin, HER-2, Vimentin, CD105, p53	ER, PR, DOG-1, CD105	ER, PR, DOG-1, CD105	chromogranin, synaptophysin, NSE, CD56, ER, PR, DOG-1, CD105

(EMA = epithelial membrane antigen; CEA = carcinoembryonic antigen; ER = estrogen receptor; MCN = mucinous cystic neoplasm; NSE = neuron specific enolase; PR = progesterone receptor).

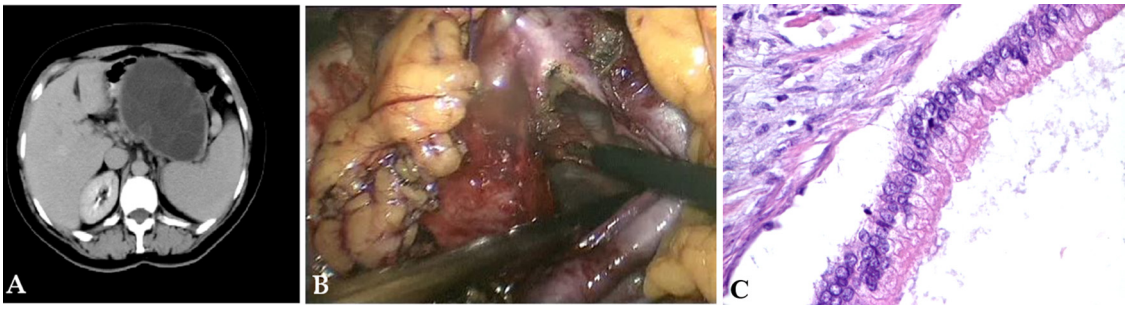

Fig. 1. The mucinous cystic neoplasm of the pancreatic tail (A) removed laparoscopically (B) is lined by tall mucin-producing epithelium with low-grade dysplasia (C).

Case 2. A 65-year-old, previously healthy male presented with an acute abdomen and a one-month history of diffuse abdominal pain. On palpation, contraction of the abdominal wall and tenderness and distension of the left hypochondrium were noted. Ultrasound examination and a CT scan of the abdominal cavity revealed a huge cystic tumor of the pancreatic tail with splenic involvement that was presumptively diagnosed as a splenic cyst. Laboratory test results fell within normal ranges.

An emergent laparotomy was performed. Intraoperative exploration of the abdominal cavity showed a large pancreaticosplenic cystic tumor with unbroken thick walls, several fissures in the splenic capsule and hemoperitoneum. Multiple white nodules were observed on the *omentum majus*, without any enlarged lymph nodes or ascites. A distal pancreatectomy, with cystic mass resection and splenectomy, was performed (en bloc excision) and three of the epiploic nodules were removed for microscopic examination.

Macroscopic examination of the surgical specimen revealed a 110 × 130 x 60 mm mucus-filled unilocular cystic tumor that involved the pancreatic tail and spleen. In cross-section, the 4–5 mm thick cystic walls had a smooth inner lining with focal papillary projections. In the pancreatic tail, adjacent to the cystic wall, a 30 × 20 × 15 mm solid tumor directly invading the adjacent spleen was also identified. No communication between the cyst and pancreatic duct was noted. The dimensions of the spleen were 190 × 80 × 40 mm (Fig. 2).

Microscopic examination revealed that the inner cystic wall was lined by tall mucin-producing epithelium with areas of low and high grade dysplasia and focally papillary projections. In the pancreatic parenchyma, solid areas with proliferations of atypical tubular structures with intraluminal mucus, embedded in a fibrous stroma, were observed. In some areas, the tumor stroma was hypercellular and expressed PR, calretinin, AE1/AE3 keratin, vimentin, SLUG and CD105 (Fig. 2). The immunoprofiles of the epithelial lining of the cyst and the malignant tubular structures and tumor stroma are presented in Tables 2 and 3.

The reactivity of the normal Langerhans cells with PR and N-cadherin and the presence in the tumor cells of vimentin, maspin, HER-2, E-cadherin, as well as the neuroendocrine markers chromogranin, synaptophysin, neuron-specific enolase and CD56 were unexpected features (Table 3). The epiploic nodules displayed a similar architecture and immunoprofile as the primary solid tumor, except for two antibodies. CD105 was not present in the tumor stroma but marked the malignant tumor cells, whereas the expression of E-cadherin was lost in the tumor cells. No lymph nodes were identified in the surgical specimen.

Based on the macro- and microscopic features, the final diagnosis

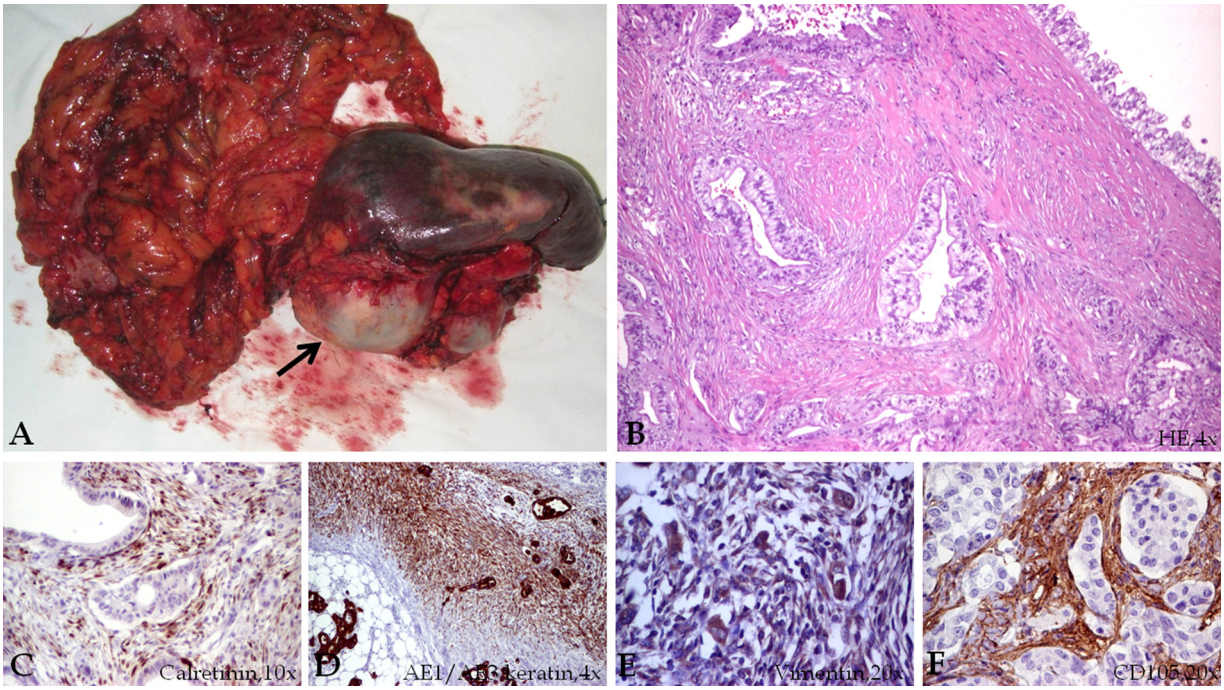

Fig. 2. The large pancreaticosplenic cystic tumor (A) is a mucinous cystic neoplasm of the pancreatic tail with invasive carcinoma (B) with malignant stroma that is marked by calretinin (C), keratin (D), vimentin (E) and CD105 (F).

Table 3
Immunoprofile of pancreatic mucinous cystic neoplasm with associated invasive carcinoma (+ refers to diffuse constant positivity and ± refers to inconstant positivity).

IHC marker	Langerhans islets	Cyst-lining benign epithelial component	Cyst-lining borderline component	Malignant epithelial component	Tumor stroma
AE1/AE3 keratin	+	+	+	+	±
EMA	+	+	+	+	−
CEA	−	−	+	±	±
Maspin	−	−	+	+	−
PR	+	±	±	±	±
ER	−	−	−	−	±
Chromogranin	+	−	−	±	−
Synaptophysin	+	−	−	±	−
NSE	+	−	±	±	−
CD56	+	−	−	±	±
HER-2	±	±	+	+	−
CD105	−	−	−	±	±
DOG-1	−	−	−	−	−
Calretinin	−	−	−	−	±
Inhibin	−	−	−	−	−
p53	−	−	±	+	−
E-cadherin	−	+	+	±	−
N-cadherin	+	−	−	±	−
SLUG	+	+	+	+	+
Vimentin	−	−	−	±	+

(EMA = epithelial membrane antigen; CEA = carcinoembryonic antigen; ER = estrogen receptor; IHC = immunohistochemical; NSE = neuron specific enolase; PR = progesterone receptor).

was “MCN of the pancreatic tail with associated invasive carcinoma and neuroendocrine differentiation, with peritoneal metastases”. The tumor was classified as pT3NxM1, respectively stage IV [12].

The patient was discharged seven days after surgical intervention with an uneventful post-operative course and normal levels of serum glucose. The patient was transfered to the Oncology Department and gemcitabine-based chemotherapy was prescribed. High pain intensity was constantly accused. The patient died at 8 months after surgery.

Case 3. A 72-year-old, non-drinking female (measuring 166 cm and weighing 52 kilos) presented with a seven-month history of inappetence, weight loss (11 kilos in seven months), modified intestinal transit (constipation) and progressively increasing epigastric and left hypochondrial pain radiating into the back. At the time of admission, a contrast-enhanced CT scan (using Iomeron 350) of the abdominal cavity revealed a 48 × 37 × 30 mm tumor of the pancreatic tail directly invading the left adrenal gland, spleen and celiac trunk, without hepatic, lung, or bone metastases or enlarged lymph nodes. Most of the laboratory test results fell within normal ranges (blood glucose 113.5 mg/dL, blood amylase 40 U/L, urinary amylase 47 U/L). Slight lymphocytosis (15.7 × 103/μL) and normocytic anemia (hemoglobin 9.1 g/dL, hematocrit 27.7%) were detected. An intraductal breast carcinoma had been surgically removed one year before without any postoperative therapy. She declared that she had smoked about two to three cigarettes per day for a period of 20 years but had ceased smoking two years previously. Apart from beta-blockers, no other medical drugs, including contraceptive pills, were used.

An explorative laparotomy confirmed that the tumor had invaded the left adrenal gland as well as the left kidney, the posterior gastric wall, mesenteric and splenic arteries and veins and the celiac trunk. It presented several epiploic metastases with no ascites. Due to the obstructive effects of the large tumor, a distal pancreatectomy, with a splenectomy and left adrenalectomy, was performed.

Gross examination of the surgical specimen revealed a 35 × 25 × 20 mm tumor of the pancreatic tail that crossed the surgical margins. The cross-section revealed a mucus-filled unilocular cyst in center of the tumor, 12 mm in diameter, with a smooth inner surface, surrounded by solid tumor mass (Fig. 3). The spleen, adrenal gland and epiploic nodules had been invaded by the tumor. The dimensions of the spleen were 70 × 35 × 30 mm.

Microscopically, the cystic wall was lined by tall mucin-producing epithelium with low-grade dysplasia and papillary projections, whereas the solid areas were similar to the second case, in terms of both pancreatic tissue and metastases. Of the six lymph nodes of the splenic hilum, one presented metastases and one was an accessory spleen with metastases. The tumor stroma had an ovarian-like aspect and expressed PR, SLUG and AE1/AE3 keratin; the tumor immunoprofile was identical to case 2 (Tables 2 and 3).

The final diagnosis was “MCN of the pancreatic tail with associated invasive carcinoma and neuroendocrine differentiation, with direct spleen and left adrenal gland invasion, with metastases in lymph nodes and accessory spleen, with peritoneal carcinomatosis. The tumor was classified as pT3N1M1, respectively stage IV.

Moderate amylasemia (116 U/L) and amylasuria (552 U/L) occurred on the first day after surgery. On the fourth day following the operation, raised levels of serum amylase were noticed (158 U/L), which remained constant in the four months following surgery. The patient was discharged seven days after surgical intervention and oncological therapy was recommended. The patient died four months after surgery.

Case 4. A 57-year-old, previously healthy male presented with a three-week history of left hypochondrial and periumbilical pain. The CT scan of the abdominal cavity revealed a cystic tumor of the pancreatic body with lymphadenopathies of the celiac axis and common hepatic artery. Laboratory test results fell within normal ranges (serum amylase 70 U/L; serum glucose 92 mg/Dl). A corporeo-caudal pancreatectomy with lymphadenectomy was performed using the LigaSure sealing system.

Macroscopic examination of the surgical specimen revealed a 120 × 30 × 5 mm tumor of the pancreatic body and tail that crossed the surgical margins. The cross-section revealed a mucus-filled unilocular cyst, a 10 mm diameter, in the center of the tumor which had a smooth inner lining and was surrounded by solid tumor mass.

Microscopic examination revealed similar features to the previous case and metastases in three of the four regional lymph nodes. Immunohistochemically, there was no keratin in the stroma even though immunoreactivity of the tumor cells for the neuroendocrine markers Chromogranin, NSE, synaptophysin and CD56 was observed. Both the dysplastic and tumor cells were marked by HER-2 (3+) and CEA expression was also observed (Fig. 4).

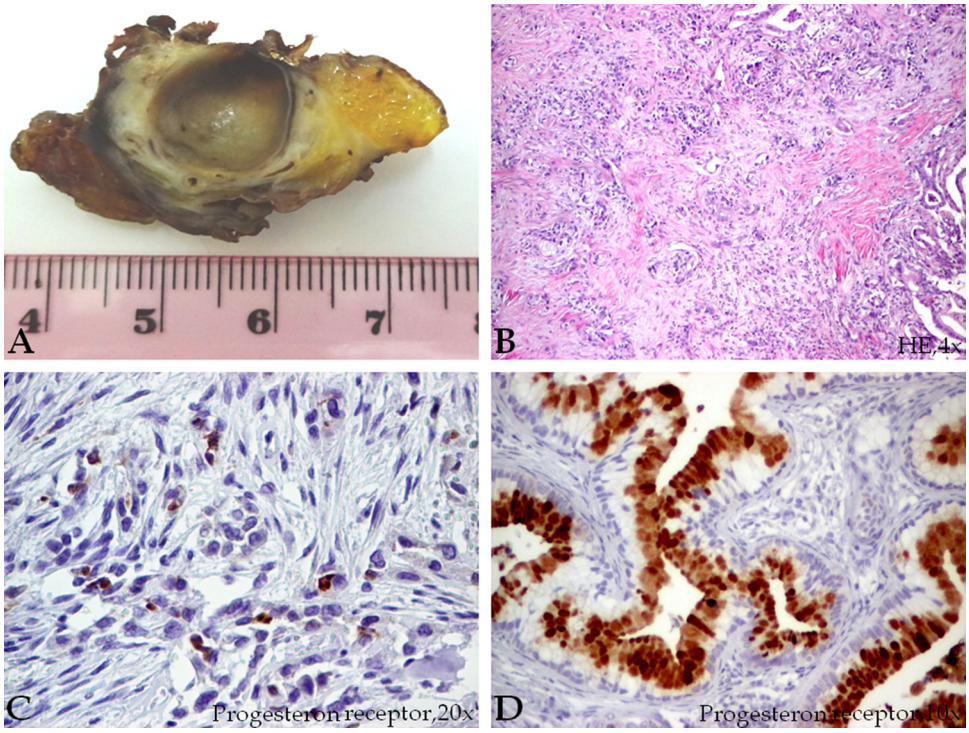

Fig. 3. The unilocular mucinous cystic neoplasm of the pancreatic tail with invasive carcinoma (A,B) has a hypercellular stroma (B) and display positivity for Progesterone receptor in both stroma (C) and tumor cells (D).

The final diagnosis was "MCN of the pancreatic tail with associated invasive carcinoma and lymph node metastases". The tumor was classified as pT3N1M0. The patient was discharged six days after surgery and there were still no complaints three months postoperatively. The patient was transfered to the Oncology department and gemcitabine-based chemotherapy was prescribed. Similar to the second case, high pain intensity was constantly accused. The patient died at 7 months after surgery.

4. Discussion

MCNs of the pancreatic tail are usually asymptomatic, being

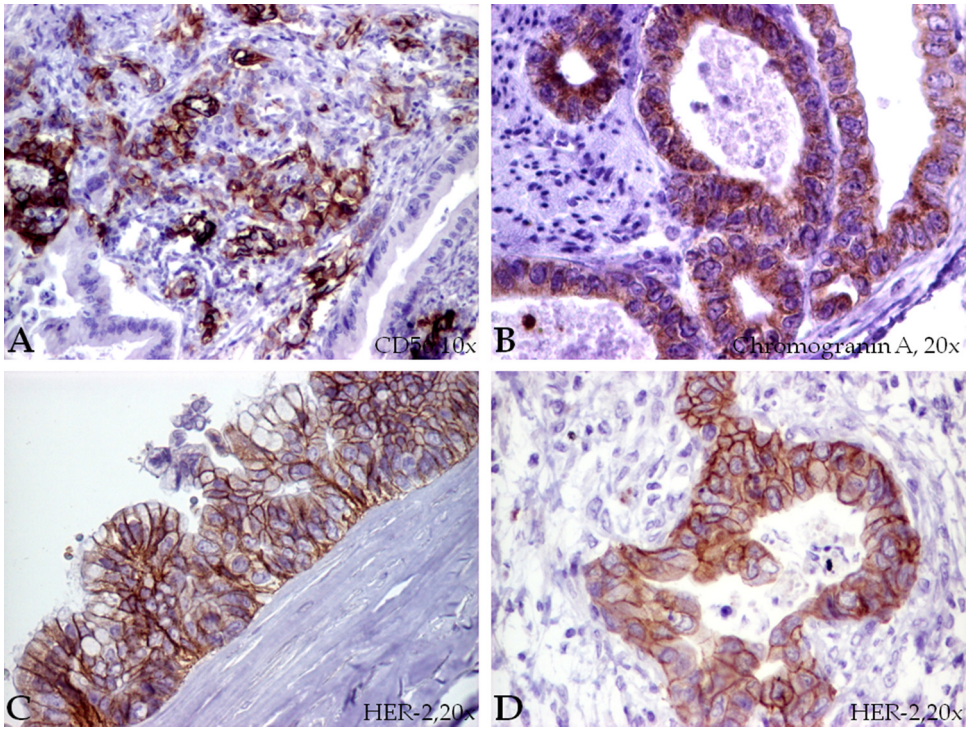

Fig. 4. The mucinous cystic neoplasm of the pancreatic tail presents neuroendocrine differentiation (A,B) and 3+ HER-2 positivity in the high-grade dysplasia (C) and invasive component (D).

incidentally discovered upon ultrasound examination or during autopsy. Large cysts can lead to compression of the stomach with associated abdominal pain and vomiting and, more rarely, compression of the splenic vein and subsequent venous congestion/thrombosis, left-sided portal hypertension, ascites and splenomegaly [9,13–17], as was observed in our second case. Associated diabetes mellitus, hemobilia and hemosuccus were also reported [9].

Pancreatitis is rarely associated and bleeding is exceptional [2,9]. Ruptured primary pancreatic MCNs associated with acute abdomen have been previously reported in 18 PubMed-cited cases, especially in pregnant women [14,15]. In some of the cases, pseudomyxoma peritonei was identified and only one of them produced splenic rupture [15]. The second case from this article is, to the best of our knowledge, the second MCN reported in the literature with spontaneous splenic rupture and subsequent hemoperitoneum, and the first case that was presented as a huge pancreaticosplenic cyst.

Regarding the therapeutic management of MCNs with high-grade dysplasia/carcinoma in situ, the Sendai and Fukuoka international guidelines advise the surgical excision of all MCNs larger than 30 mm in diameter and of all tumors with thick septa, intramural solid nodules and eggshell calcifications, independent of their diameter [2,3,6,7,10]. A serum level of CA 19-9 higher than > 37 U/mL is an indicator of malignancy, with a positive predictive value rate of 95.7% and a 35.8% sensitivity rate [10].

It was estimated that the rate of growth is about 20 mm per year and the time taken to progress from a benign to a malignant MCN is about 10 years [1,2,9]. In pregnant women, the evolution is more rapid, four months being enough to double the tumor size [16]. The five-year survival rate is 100% in benign MCNs and about 44% in their invasive forms [2,12]. Although laparoscopic resection can be performed on MCNs with an associated invasive component [5–7], the risk of a remnant solid component and an incorrect or non-specific preoperative diagnosis in two thirds of cases [5], means that surgery is advisable. In our first case, based on the solid mass described at CT sac, an open surgery was indicated but the patient refused the intervention. However, as she is still alive at two years after surgery, the solid component was probably a fibrotic focus of chronic pancreatitis.

Regarding the histogenesis of MCNs, based on the fact that they predominantly occur in females and ovarian-like stroma that test positive for ER in 23% and PR in 71% of cases [16], it was even suggested that yolk sac cells or primitive germ cells have migrated to the pancreas during embryogenesis due to the close proximity of the dorsal/distal pancreas to the primordial gonad, or that the endodermal immature stroma is stimulated by female hormones [14,16].

Based on the fact that 6.5% of MCNs are associated with synchronous or metachronous breast tumors [2], as in our second case, and that 20 cases were reported in the literature between 1968–2015 as being activated during pregnancy, it is supposed that the female sex hormones influence the behavior of MCNs [2,7,10,16].

The significance of Langerhans cells testing positively for PR, as well as similarities between the stroma of the fetal pancreas and the MCN, remains unclear. In this paper, the simultaneous presence of PR, N-cadherin and neuroendocrine markers in Langerhans islets and malignant tumor cells could indicate a mixed MCN with exo- and endocrine components, this histological subtype being as of yet unreported in the literature. A previously published article identified scattered neuroendocrine cells – testing positive for chromogranin and synaptophysin – in the benign epithelial cells of one MCN with malignant stroma (a variant of MCN known to be aggressive), but not in the malignant component. This was similar to two of our cases (two and three) [18]. The simultaneous presence of CD105 and keratin in the stromal cells can indicate a mesenchymal-to-epithelial-endothelial transition of the stromal cells that could increase the aggressiveness of the tumor. In cases two and three, which had malignant stroma, the presence of vimentin in the tumor cells indicated the EMT. In the epiploic nodules, the decrease in E-cadherin confirmed the EMT of the malignant component [19].

In conclusions, four unusual MCNs with aggressive behavior and normal amylasemia and amylasuria were described in this report. In the first case, presumptively diagnosed as a hydatid cyst, the laparoscopic resection was followed by the identification of a remnant solid malignant component three months post-surgery that proved to be a fibrotic foci. In the second case, MCNs with invasive carcinoma caused splenic congestion and rupture. Moreover, in the second and third cases, the neuroendocrine differentiation and EMT observed in the tumor cells was associated with the presence of a combination of keratin/CD105 in the stromal cells. MCNs with neuroendocrine differentiation, EMT and malignant CD105-positive stroma could be a new histological entity with aggressive behavior, whereas the presence of HER-2 could be used as a further indicator for a possible answer in trastuzumab therapy.

Conflict of interest

None declared.

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