CASE REPORT

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A nonfunctional neuroendocrine tumor of the pancreas – a case report

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Abstract

Pancreatic neuroendocrine tumors (pNETs) represent about 1–5% of the pancreatic tumors, having an annual incidence of about 1/100 000, with difficult positive and differential diagnostic, especially in nonfunctioning tumors. We present a case of large dimensions (10/8 cm) pancreatic tail NET developed in a 65-year-old woman, incidentally discovered while she was examined for a strangled inguinal hernia. The patient had no specific tumor signs and the imagistic examination did not reveal any metastases. The histopathological and especially the immunohistochemical examinations were decisive for the diagnosis, treatment and prognostic establishment. The tumor showed intense positivity for chromogranin, synaptophysin, CD56, CD117, CK19, MNF-116 and negative for CK5/6 and CK7. The proliferative index established through the Ki-67 assessment was around 3%, while p53 was positive in 25–30% of tumor cells nuclei.

Keywords: pancreas, neuroendocrine tumors, immunohistochemistry, chromogranin, synaptophysin.

Introduction

Pancreatic neuroendocrine tumors (pNETs) are relatively rare tumors when compared to pancreatic cancer, a devastating illness [1] with a five year survival rate of about 5–6% [2–4], when compared with the cancer overall five year survival rate of 67%, or other cancer survival rates, that, in some cases, rise above 90% [5]. This very aggressive behavior is due to some anatomic, but mostly biological features [6] that place pancreatic cancers on the 4th place in cancer related deaths list, with continuous rising incidence and mortality [7]. Some authors estimate that by 2030, the pancreatic cancer will be the second cancer related death cause, just after pulmonary cancer, if the strategies for prevention or early diagnosis will fail to be implemented [8].

In a larger context of pancreatic tumors, pancreatic neuroendocrine tumors represent 1–5%, arising from pancreatic islet cells (endocrine pancreas), can have a benign or malignant behavior, but either way they have a better prognosis than pancreatic adenocarcinomas [9, 10]. About 1000 new pNETs are diagnosed in USA every year [9]. These tumors prevalence and the incidence increased in last three decades without any clear explanation [11, 12]. The clinical diagnosis of those tumors is usually difficult, because the signs and symptoms are either absent in the presence of nonfunctional tumors, or very complex, due to polypeptide released by the functional tumors.

We report a case of a large pNET discovered during a clinical examination of the abdomen in a female patient presented in emergency conditions for strangled right femoral hernia, which was manually reduced in the emergency room.

Case report

The 65-year-old female patient presented on March 21, 2012 in the Emergency Room of the Emergency County Hospital of Craiova, Romania, for a painful tumor, suddenly appeared in the right Scarpa triangle about two hours before, during an intense effort. The patient also reported abdominal discontinuous pain associated with bowel movements, nausea and vomiting and the absence of intestinal transit. The clinical examination confirmed the presence of a 3 cm right femoral hernia and managed to reduce it in the abdominal cavity with remission of clinical complaints. While monitoring the patient, the repeated

clinical examination identified a persistent tumor localized at the limit of epigastric area with left hypochondrium. The tumor was estimated at about 6/8 cm, firm, relatively well delineated, fixed on the posterior planes, but without infiltration of the superior planes or anterior abdominal wall.

The patient was subjected to ultrasonography that discovered an isoechoic parenchymatous tumor of 6/7.5cm, located between the spleen, left kidney and the body of the pancreas. The tumor appeared relatively well delineated, with a heterogeneous structure, having the same vascular density as the pancreatic tissue, without any local or paraaortic lymph nodes enlargement. There were no signs of metastases in other abdominal organs.

The CT scan confirmed the tumor site as the tail of the pancreas, well delineated, heterogeneous, having the same density as the pancreatic tissue, adherent to splenic vein (Figure 1). Thoracic or abdominal metastases were also absent on CT scan, as well as signs of nodal involvement.

The main biological parameters were hemoglobin 14 g/dL; blood glucose 100 mg/dL; urea 34 mg/dL; white blood count (WBC) 6500/mm^2; thrombocytes 197 000/mm^2, while bilirubin, TGO, TGP, electrolytes, coagulation tests were within normal limits. The seric values for chromogranin A (ELISA) 26 μg/L (normal 27–94 μg/L), for serotonin 103 μg/L (normal 80–400 μg/L), and for 5-hydroxyindolacetic acid 3.8 mg/24 h (normal 2–8 mg/24 h).

Since an echo-endoscopy with transgastric fine-needle aspiration was not available, after obtaining the informed consent, the patient was subjected to exploratory laparotomy with tumor removal intent. After xipho-subumbilical laparotomy, during the exploratory time, we identified a 10/8 cm pancreatic tumor confined to body and tail, without any major adherence or involvement to the adjacent organs. However, the splenic vein and the left portion of the mesocolon were closely attached to it (Figures 2 and 3). There were no identifiable metastases or lymph node involvement in the peritoneal cavity, organs or retro-peritoneum. We decided and performed a caudal pancreatectomy with en-block spleen removal. The Wirsung canal was individually legated, followed by the pancreatic stump suture and vicinity drainage. The femoral defect was sutured after the hernia sac dissection and removal. The postoperative evolution was simple, without any complications and a rapid patient recovery that was discharged six days later.

Four weeks after the surgical resection, due to the clinical presentation, morphological and especially immunohistochemical (IHC) characteristics that will be further discussed, the patient entered a chemotherapy protocol with Everolimus (Afinitor®, Novartis), 5 mg/day *p.o.* in continuous four weeks administration for six cycles. The follow-up included clinical examination, biological and ultrasonography investigation every 30 days, while abdominal CT was performed first after six months and then every year since surgery, without revealing any signs of recurrence or distant metastases.

Following macroscopic analysis and digital imaging of the specimen, fragments from the tumor, including resection margins, were taken for the histopathological and immunohistochemical diagnosis. The fragments were fixed in 10% neutral formalin solution for 72 hours and embedded in paraffin, using the classical protocol. The sectioning was performed using a Microm HM350 rotary microtome, equipped with a transfer system of the sections in a water bath (STS, Microm). The 4 μm-thick histological cups were stained with Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichromic.

For the immunohistochemical study, the histological sections were collected on poly-L-Lysine coated slides in order to increase adhesion of biological material on a histological blade. The blades were incubated in a thermostat at 37^{0}C for 24 hours, and then the sections were dewaxed and hydrated. Antigen unmasking was performed by boiling sections in a sodium citrate solution, pH 6, for 21 minutes in a microwave oven (seven cycles of 3 minutes). The endogenous peroxidase activity was blocked by placing the histological sections in 3% hydrogen peroxide for 30 minutes, at room temperature, followed by washing in distilled water for 10 minutes and a wash in 1% phosphate-buffered saline (PBS) solution for 5 minutes. The non-specific sites were blocked by passing the histological sections through 2% skim milk for 30 minutes.

The sections were then incubated with primary antibodies for 18 hours (overnight) in a refrigerator at 4^{0}C. The next day, a biotinylated secondary antibody was applied for 30 minutes at room temperature, after which washing was carried out in 1% PBS (three baths of 5 minutes). Streptavidin–HRP was applied for 30 minutes at room temperature, followed by washing the slides in 1% PBS (3×5 minutes). The signal was detected using 3,3'-Diaminobenzidine (DAB) (Dako) and the reaction was stopped under microscopic control using 1% PBS, when the IHC signal was optimal, thus avoiding overstaining the histological sections. The contrast was obtained with Mayer's Hematoxylin, followed by alcohol dehydration, xylene clarifying and blades mounting using DPX medium (Fluka).

For the positive and differential diagnosis, we used the following IHC markers (Table 1):

Table 1 – ***Characteristics of the antibodies used in this study***

Antibody	Code	Clone	Antigen retrieval	Dilution	Source
Ki-67	M 7240	MIB-1	EDTA, pH 9	1:50	Dako
p53	M 7001	DO-7	Sodium citrate buffer, pH 6	1:50	Dako
Chromogranin	ab15160	polyclonal	Sodium citrate buffer, pH 6	1:250	Abcam
Synaptophysin	ab32127	YE269	Tris-EDTA buffer, pH 9	1:400	Abcam
Cytokeratin 5/6	M 7237	D5/16 B4	EDTA, pH 8	1:50	Dako
Cytokeratin 7	M 7018	CV-TL 12/30	Sodium citrate buffer, pH 5	1:100	Dako

Antibody	Code	Clone	Antigen retrieval	Dilution	Source
Cytokeratin 19	M 0888	RCK 108	EDTA, pH 8	1:50	Dako
Cytokeratin MNF-116	M 0821	MNF-116	EDTA, pH 8	1.100	Dako
CD34	ab81289	EP373Y	Sodium citrate buffer, pH 6	1:100	Abcam
CD56	M7304	123C3	Sodium citrate buffer, pH 5	1:250	Dako
CD117	A 4502	polyclonal	Sodium citrate buffer, pH 5	1:400	Dako

The histopathological examination showed a trabecular pattern parenchymatous tumor, with rich collagenous stroma. Tumor cells presented a medium size, with a fine granularly acidophil cytoplasm, and a large nucleus, with 2–3 visible nucleoli and a grained chromatin, with a "pepper salt" aspect (Figures 4 and 5). The GS trichromic staining showed that the tumor did not overreached the pancreatic capsule (Figure 6), and its stroma was formed of collagen fibers, organized in conjunctive septa, with a plexiform arrangement, and lot of blood vessels (Figure 7). According to the microscopic aspect, the tumor was considered as primitive neuroendocrine tumor of the pancreas.

For the positive and differential diagnosis, there were performed various immunohistochemical stainings. The tumor cells reaction to the anti-chromogranin and anti-synaptophysin antibodies was quite an intense one (Figures 8 and 9), thus confirming the neuroendocrine origin of the tumor. However, the intensity of the immunohistochemical reaction, both to chromogranin and synaptophysin, was not a homogenous one, areas with a strongly intense reaction coexisting with areas with a poor reaction to the two immunohistochemical markers. Also, there were identified rare cells with a negative reaction to the two antibodies.

For highlighting the neuroendocrine origin, we investigated the tumor cells reaction to the CD56 antibody, knowing the fact that this marker couples with a neural cell adhesion molecule present on the surface of neurons and neuroendocrine cells. The reaction of tumoral cells to CD56 had a medium intensity, being present both in the peripheral cytoplasm and in the intercellular space (Figure 10), thus confirming their neuroendocrine origin.

The proliferative activity of tumor cells was investigated by using the Ki-67 and p53 antibodies. In our case, less than 3% of the tumor cells nuclei were positive to Ki-67 (Figure 11). Instead, the p53 antibody was a lot more intense, around 25–30% of tumor cells being marked by this antibody (Figure 12).

The analysis of the presence of certain cytokines in the tumor cells cytoplasm showed contradictory results. The immunohistochemical reaction was negative for the cytokeratins 5/6 and 7 (Figures 13 and 14), but it was intensely positive for cytokeratin 19 and MNF-116 (Figures 15 and 16).

Another immunohistochemical marker investigated by us was CD117, known as a marker involved in the cellular proliferation, differentiation and survival. As it may be observed (Figure 17), the reaction of tumor cells to CD117 was positive in most of the cells, especially in the peripheral cytoplasm.

The selective highlighting of blood vessels was performed by using the CD34 antibody, which marks the endothelial cells. There was observed that the tumor presented a well-developed network of blood vessels, especially arterioles and capillaries, which inosculated with each other (Figure 18). High caliber vessels were present in the structure of conjunctive septa from the tumor stroma.

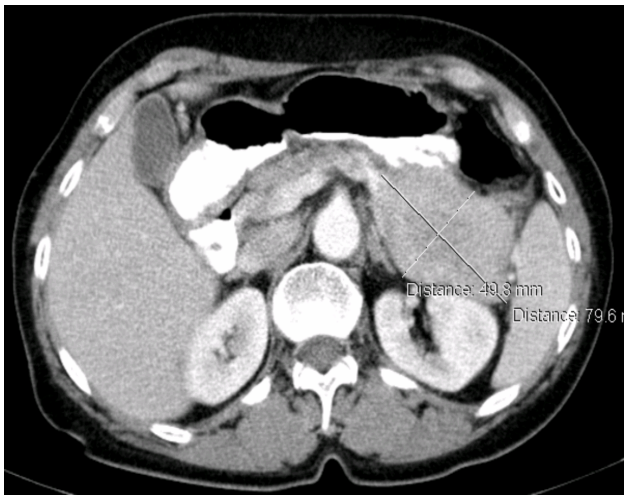

Figure 1 – ***CT scan image of the abdomen, highlighting at pancreas tail the presence of a 79.6/49.8 mm tumor, with a relatively clear contour, a discrete heterogeneous structure, reaching the spleen hilum and covering the anterior side of the left kidney.***

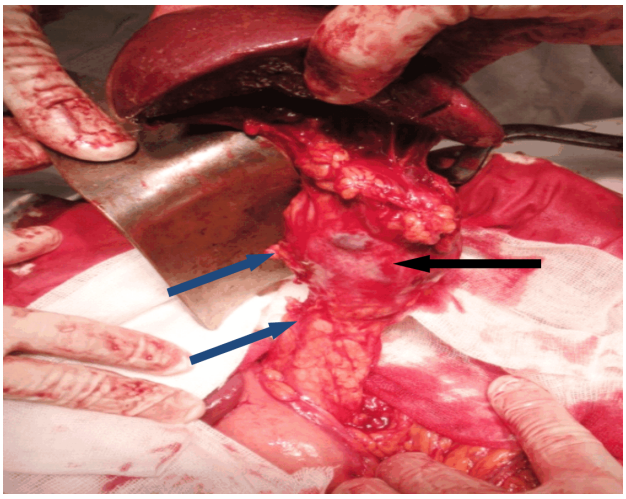

Figure 2 – ***Intraoperative aspect with spleen and pancreas tail mobilized; black arrow – tumor; blue arrows – ligated and transected splenic artery and vein; in the distal part, the vein passes through the tumor while the artery is intimately adherent to it.***

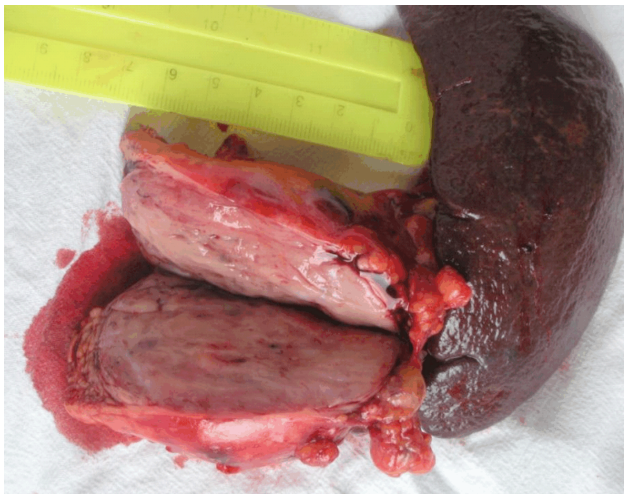

Figure 3 – *Macroscopic aspect of the pancreatic tumor: tumor with a parenchymatous aspect of about 10/8 cm, well differentiated, with a heterogeneous structure and small hemorrhagic areas.*

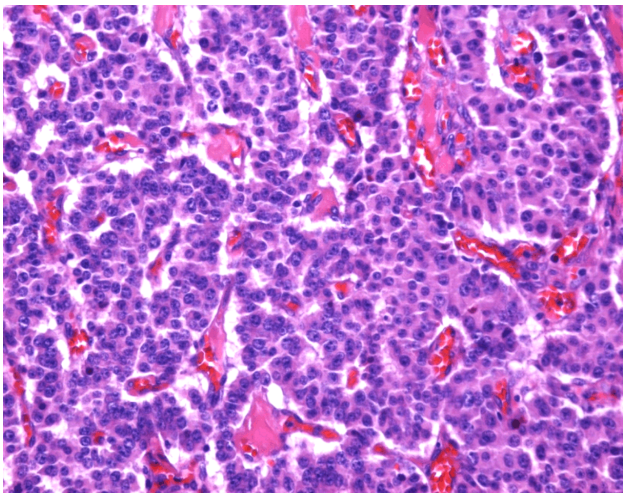

Figure 4 – *Overall microscopic aspect of the tumor, made up of medium sized cell cordons, divided by fine conjunctive septa, rich in blood vessels. HE staining, ×200.*

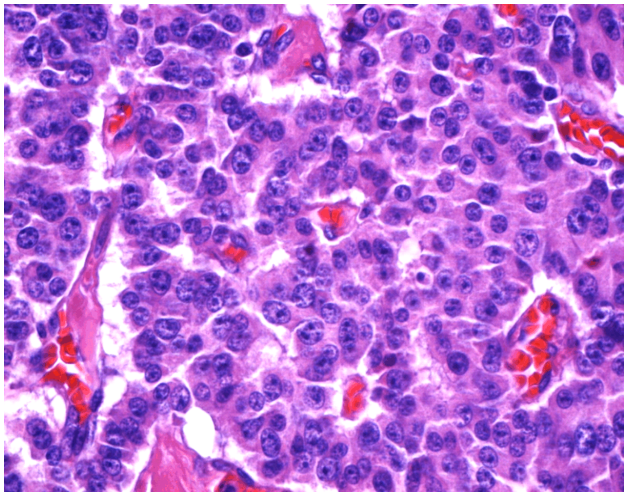

Figure 5 – *Tumor cells with acidophilic cytoplasm, finely granular, large nuclei, with clear nucleoli and chromatin heterogeneously distributed, leaving the impression of "salt and pepper". HE staining, ×400.*

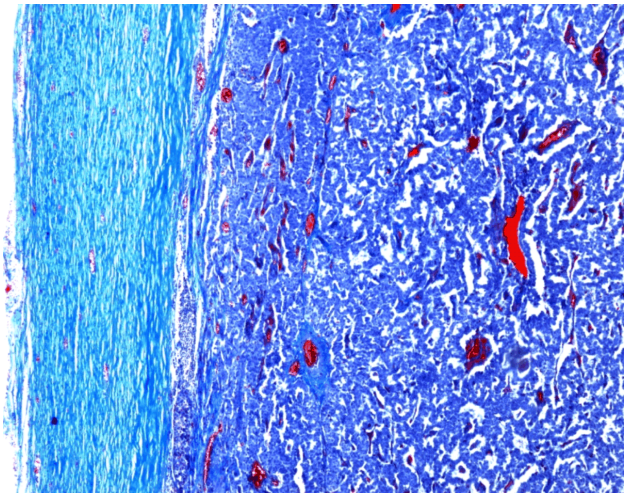

Figure 6 – *Microscopic image of the pancreatic capsule that appears intact. GS trichromic staining, ×40.*

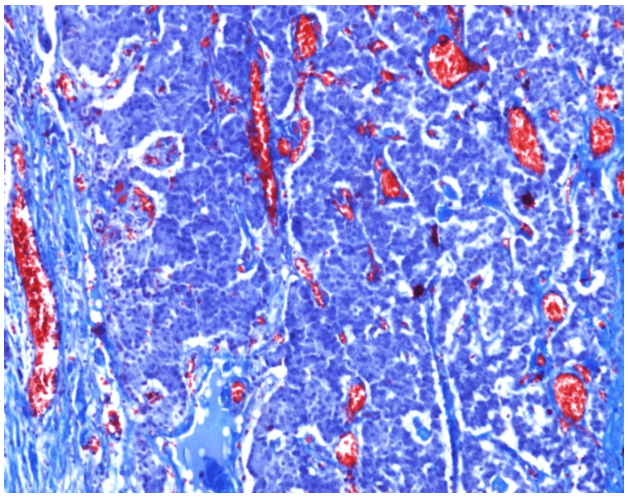

Figure 7– *Highly vascularized tumor stroma. GS trichromic staining, ×100.*

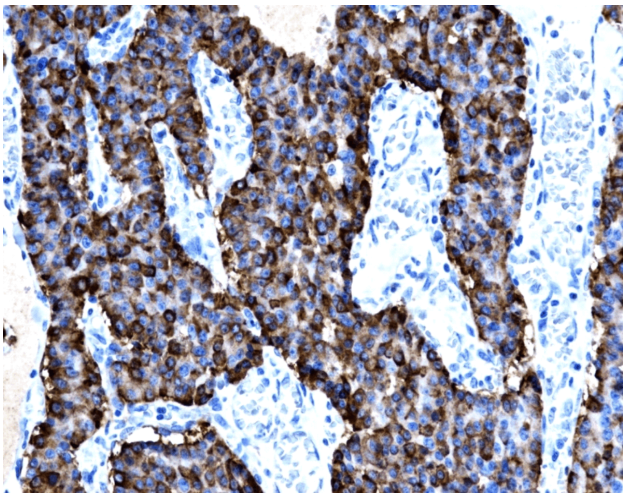

Figure 8 – *Tumor cells with an intensely reactive cytoplasm to the anti-chromogranin antibody. Immunomarking with the anti-chromogranin antibody, ×200.*

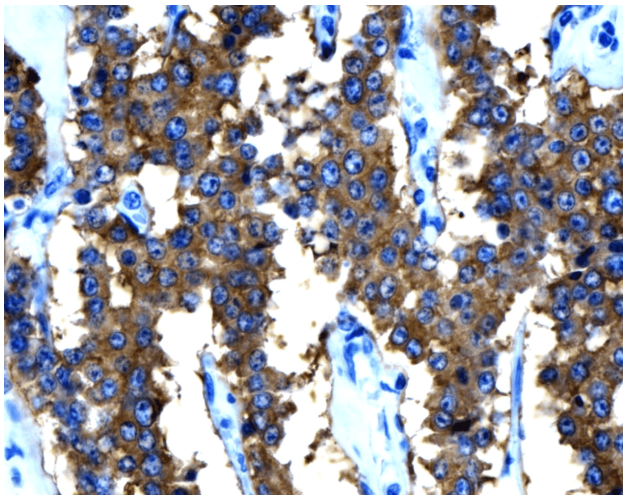

Figure 9 – ***Intense reaction of tumor cells to synaptophysin. Immunomarking with anti-synaptophysin antibody, ×200.***

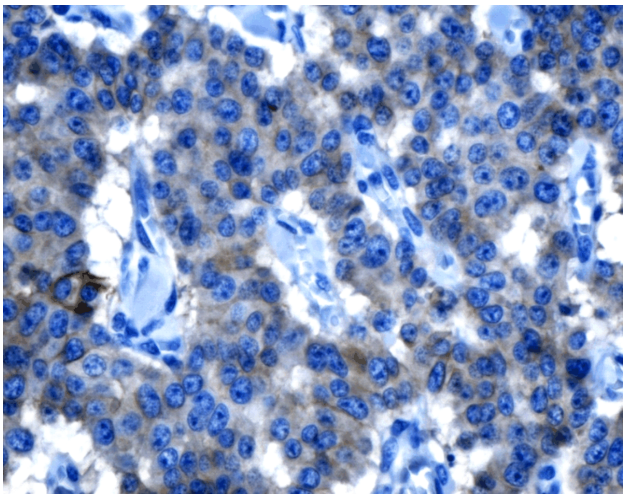

Figure 10 – ***Tumor parenchyma with a medium intense reaction to CD56 in peripheral cytoplasm and in the intercellular space. Immunomarking with anti-CD56 antibody, ×400.***

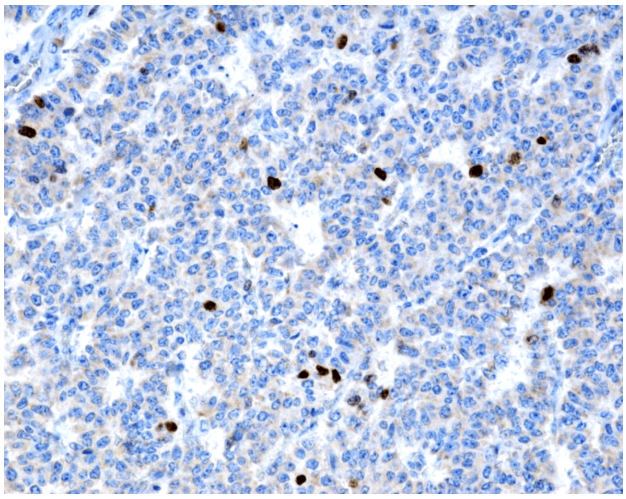

Figure 11 – ***Microscopic image showing that less than 3% of tumor cells are positive to the Ki-67 antibody. Immunomarking with the anti-Ki-67 antibody, ×200.***

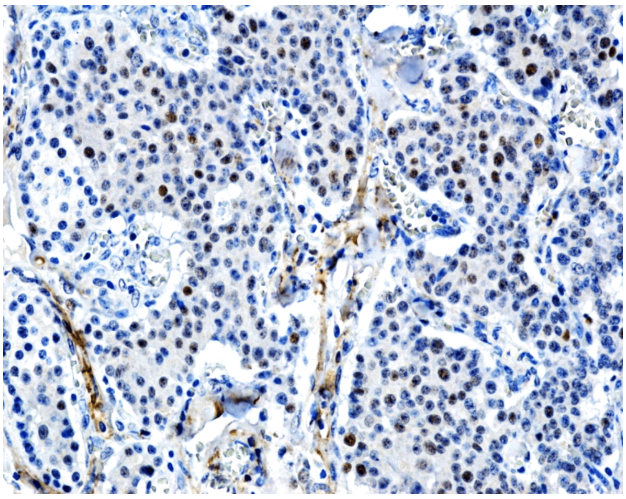

Figure 12 – ***Intense reaction to the p53 antibody in about 25–30% of tumor cells. Immunomarking with the anti-p53 antibody, ×200.***

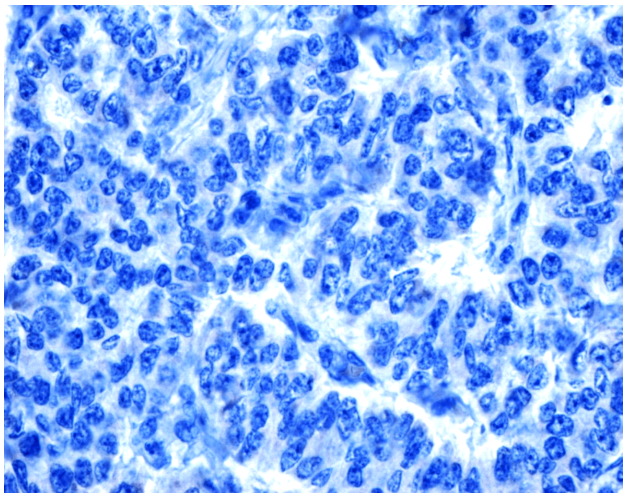

Figure 13 – ***Negative reaction of tumor cells to CK5/6. Immunomarking with the anti-CK5/6 antibody, ×400.***

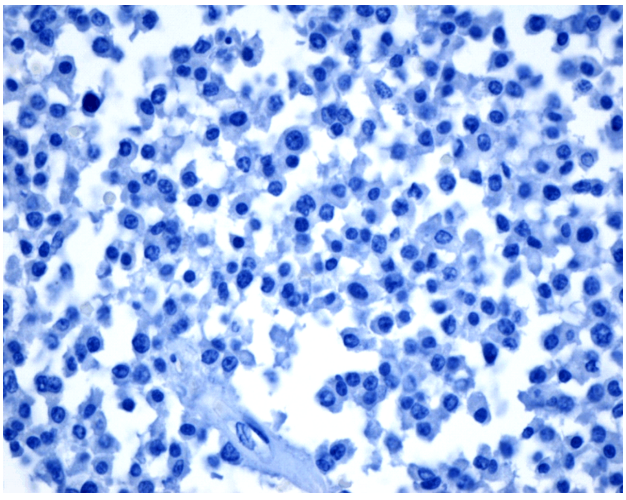

Figure 14 – ***Tumor cells with a negative reaction to CK7. Immunomarking with the anti-CK7 antibody, ×200.***

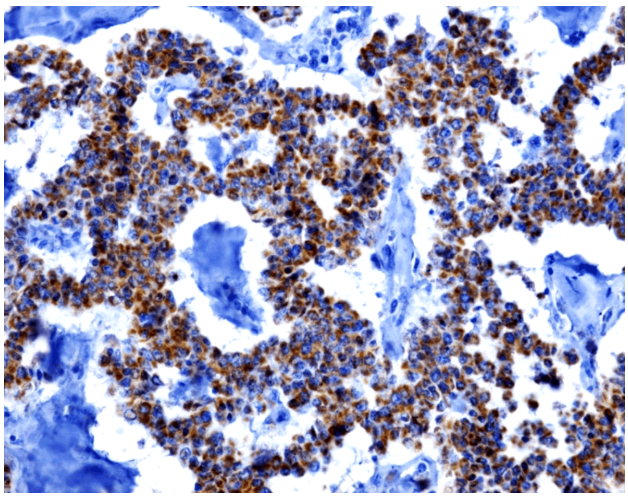

Figure 15 – ***Intense reaction of tumor cells to CK19. Immunomarking with the anti-CK19 antibody, ×200.***

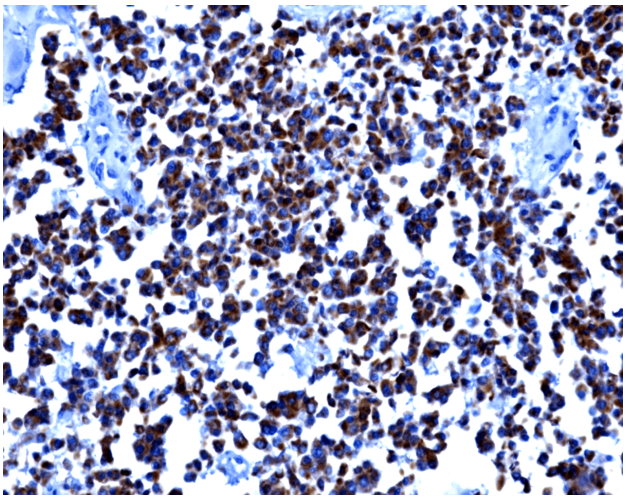

Figure 16 – ***Tumor cells intensely positive to the cytokeratin MNF-116. Immunomarking with the anti-CK MNF-116 antibody, ×200.***

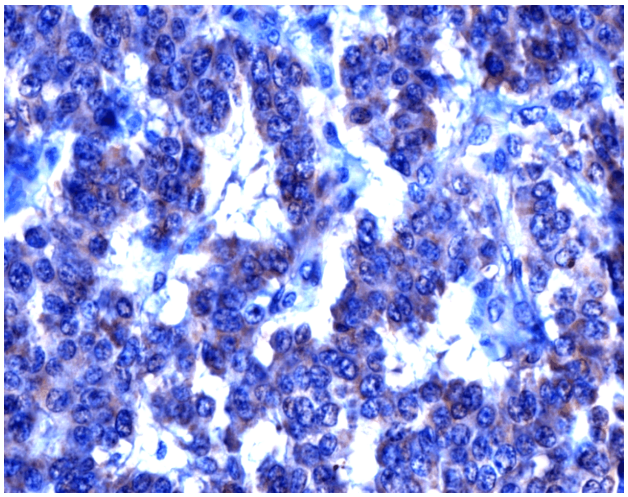

Figure 17 – ***Tumor cells almost completely marked by the CD117 antibody. Immunomarking with the anti-CD117 antibody, ×400.***

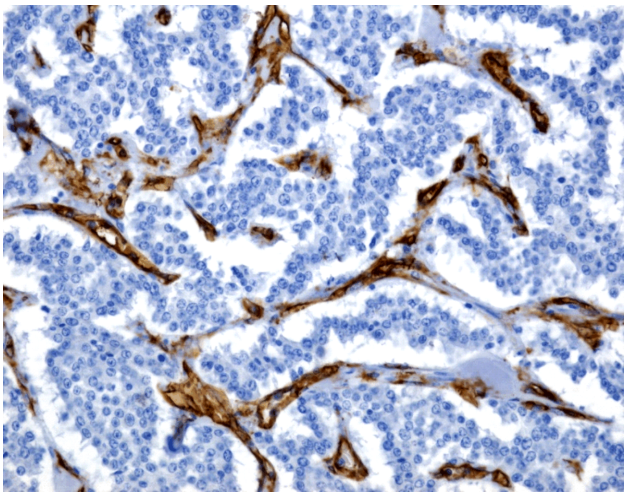

Figure 18 – ***Dense reaction of anastomosed microvessels, present both in the stroma, as well as in the tumor parenchyma. Immunomarking with the anti-CD34 antibody, ×200.***

Discussion

The pancreas is the most frequent site for the development of neuroendocrine tumors at the digestive tract level [13]. These tumors aggressiveness is quite variable, from almost benign to extremely aggressive ones, most of them being moderately malignant [14, 15]. According to the *WHO* classification in 2010, the neuroendocrine tumors of the digestive system, including the pancreatic ones, are divided into well-differentiated neuroendocrine tumors (1st stage), which have a benign or uncertain malignant behavior, well-differentiated neuroendocrine carcinomas (2nd stage), which are characterized by a low malignity degree, and poorly-differentiated neuroendocrine carcinomas (3rd stage) (usually small-cell carcinomas), with a high malignity degree [16–19].

Another classification divides pancreatic neuroendocrine tumors, according to the quantity of released hormones, into functional and non-functional types [20–22]. Functional tumors secrete in the blood flow hormones, such as insulin, gastrin, glucagon, the vasointestinal polypeptide (VIP), somatostatin or other polypeptides, many times in high quantities, leading to more or less serious symptoms, thus allowing the clinical and paraclinical diagnosis. The most frequent functional pancreatic neuroendocrine tumors are insulinomas and gastrinomas [15, 23].

Non-functional neuroendocrine tumors do not secrete the hormones in an adequate quantity as to give clear clinical symptoms. From this reason, non-functional pancreatic tumors are, most of the time, diagnosed only after the cancer metastasizes in other organs [24], or they are accidentally discovered, during some medical imagistic examinations or at necropsy [25, 26]. According to some studies, most of the pancreatic neuroendocrine tumors are functional, only 15% being non-functional ones [27].

In our case, the discovery of the pancreatic tumor was purely accidental, during clinical examination for a strangled femoral hernia, followed by echography and CT scan of the abdomen. Although the tumor sizes were quite large, there were no metastases either at diagnosis time or after three years since tumor removal. The fact that it did not present any clinical symptoms, and the seric values of glycemia and chromogranin were within

normal values, we considered that it was a non-functional neuroendocrine tumor. The peculiarity of our case consisted in the relatively large size of the tumor (10/8 cm), absence of metastases and favorable post-treatment evolution.

It is well-known the fact that most of the pancreatic neuroendocrine tumors have a malignant potential [28, 29], especially non-functional ones. The larger the tumor is, the less favorable the prognosis, as they give liver and ganglion metastases [30]. One of the reason for which the patient received adjuvant targeted oncological therapy was the tumor size and vascular invasion (Figure 2) that pleaded for malignancy as the tumor was staged T4 (tumor over 4 cm invading adjacent organs or the wall of large vessels) according to *European Neuroendocrine Tumor Society* (ENETS).

Most of the pancreatic neuroendocrine tumors are diagnosed in patients over 40-year-old [31, 32], although they may also appear at any age [12, 33]. The diagnosis of pancreatic tumor was made, in our case, in a person over 65-year-old, without any clinical symptoms, through clinical exam and some medical imagistic investigations for another medical condition.

The histopathological and immunohistochemical examinations play an essential role in establishing the diagnosis and prognosis of a neuroendocrine tumor. Most of the neuroendocrine tumors are more or less intensely reactive to chromogranin and synaptophysin. The chromogranin A is a glycoprotein with a molecular weight of 46 kDa, physiologically present in all neuron types, and also in the neuroendocrine tumor cells [34–36]. Numerous studies in the last 20 years have confirmed that chromogranin is a reliable marker for the positive and differential diagnosis of neuroendocrine tumors, including of the gastroenteropancreatic ones [37–41]. Synaptophysin is a glycoprotein with a molecular weight of 38 kDa, present in almost all neurons of the central nervous system, in the neuroendocrine cells, but also in the tumor cells of neuroendocrine tumors, being an essential marker for this type of tumors [42, 43]. In our study, the tumor cells were intensely positive to chromogranin and synaptophysin.

Other markers used by us for the assessment of the tumor neuroendocrine characteristic were CD56 and CD117; both markers presented an intense reaction in most tumor cells. Numerous studies showed that the neuroendocrine tumors of the digestive tract express a positive reaction to the CD56 antibody [27, 44]. Choi *et al.* (2013) presented a case of non-functional pancreas neuroendocrine tumor complicated with multiple heart metastases, where the immunostaining of tumor cells with CD56 was intensely positive [45]. We should note that CD56 is also positive in another rare tumoral entity of the pancreas that occur mostly in women – solid pseudopapillary neoplasms, that in some cases are positive for synaptophysin but always negative for chromogranin [46]. Instead, the CD117 reaction is variable and controversial. It was found positive in lung carcinomas, but negative in gastrointestinal endocrine tumors [47].

For assessing the cellular proliferation index, we used the Ki-67 antibody that marks a nuclear protein associated to cellular proliferation. By assessing the reaction to this immunomarker, we observed that less than 3% of the tumor cells nuclei presented a positive reaction, which suggested that the tumor was a well-differentiated one, with a good prognosis. Multiple studies suggested that Ki-67 is one of the most precise parameters regarding the aggression of pancreatic and gastrointestinal neuroendocrine tumors [48–51]. Since 2010, the *WHO* experts included the Ki-67 immunomarker on the list of parameters characterizing the biological behavior of pancreas neuroendocrine tumors, together with the tumor size, neighboring organs infiltration, presence of remote metastases, presence of adeno- or angiopathies, or neuroinvasion [19, 42, 52, 53]. According to these experts, pancreatic neuroendocrine tumors, with a Ki-67 index lower than 20% are well-differentiated tumors, with a better prognosis than the tumors with a Ki-67 index higher than 20%, which are poorly differentiated, having a reserved prognosis.

Another immunohistochemical marker investigated by us was protein p53, the synthesis product of TP53 gene, also known as the "genome guardian", having the role of regulating the cellular increase and division, by inducing the cellular apoptosis to the cells with irreversible genetic alterations. In our case, about 25–30% of the tumor cells nuclei were positive to the p53 antibody. The increase of p53 positivity is due to the presence of certain nuclear aberrations that led to the emergence of some excessive mutant p53 proteins [54]. Some studies showed that, in gastroenteropancreatic tumors, they had an intensely positive reaction to p53, up to 49% [55]. Other studies suggested that the p53 protein expression is correlated to the stage of differentiation of neuroendocrine tumors [19].

The cytokeratins assessment in tumor cells showed that basic cytokeratins CK5/6 and 7 were negative, while CK19 and MNF-116 were intensely positive. Similar results were reported in other studies, as well [42, 56].

Regarding the tumor vascularization, we showed, both with classically stained histopathological samples (with HE or GS trichromic), and by using the CD34 antibody, that the tumor contained a well-developed network of blood vessels, especially low caliber vessels. According to some authors, large, non-secretant neuroendocrine tumors are hypervascularized [57, 58]. It is well-known that the more vascularized the tumor is, the higher the risk for early metastases is. In our case, even though the tumor had quite large sizes at diagnosis time, with a well-developed vascularization, there were no metastases present.

Conclusions

Pancreatic neuroendocrine tumors are rare tumors of the pancreas that can have a benign or malignant behavior. For non-functional tumors, the diagnostic is difficult since the general symptoms are absent. Our case highlights the importance of careful clinical exam seconded by imagistic tests that can raise the diagnostic suspicion. For positive and differential diagnostic, prognostic and management of such cases IHC stainings are crucial, especially chromogranin and synaptophysin, CD56, CD117, CK19, Ki-67 and p53. Although, in our case, there were some criteria pleading for malignancy as tumor size and vascular invasion that justified oncologic adjuvant treatment, the lack of metastases at presentation and low Ki-67 index

in combination with complete surgical removal of the tumor confers a good prognostic, since the patient does not show any signs of recurrence or metastases at three years follow-up.

Conflict of interests

The authors declare that they have no conflict of interests.

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References

[1] Ansari D, Gustafsson A, Andersson R. Update on the management of pancreatic cancer: surgery is not enough. World J Gastroenterol, 2015, 21(11):3157–3165.
[2] Hidalgo M. Pancreatic cancer. N Engl J Med, 2010, 362(17): 1605–1617.
[3] Feig C, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. Clin Cancer Res, 2012, 18(16):4266–4276.
[4] Hartley ML, Bade NA, Prins PA, Ampie L, Marshall JL. Pancreatic cancer, treatment options, and GI-4000. Hum Vaccin Immunother, 2014, 10(11):3347–3353.
[5] American Cancer Society. Cancer Facts and Figures 2012. http://www.cancer.org/acs/groups/content/@epidemiology surveilance/documents/document/acspc-031941.pdf.
[6] Wood LD, Hruban RH. Pathology and molecular genetics of pancreatic neoplasms. Cancer J, 2012, 18:492–501.
[7] Eheman C, Henley SJ, Ballard-Barbash R, Jacobs EJ, Schymura MJ, Noone AM, Pan L, Anderson RN, Fulton JE, Kohler BA, Jemal A, Ward E, Plescia M, Ries LA, Edwards BK. Annual Report to the Nation on the status of cancer, 1975–2008, featuring cancers associated with excess weight and lack of sufficient physical activity. Cancer, 2012, 118(9):2338–2366.
[8] Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res, 2014, 74(11): 2913–2921.
[9] Landry CS, Cavaness K, Celinski S, Preskitt J. Biochemical prognostic indicators for pancreatic neuroendocrine tumors and small bowel neuroendocrine tumors. Gland Surg, 2014, 3(4):215–218.
[10] McKenna LR, Edil BH. Update on pancreatic neuroendocrine tumors. Gland Surg, 2014, 3(4):258–275.
[11] Lawrence B, Gustafsson BI, Chan A, Svejda B, Kidd M, Modlin IM. The epidemiology of gastroenteropancreatic neuroendocrine tumors. Endocrinol Metab Clin North Am, 2011, 40(1):1–18, *vii*.
[12] Öberg K, Knigge U, Kwekkeboom D, Perren A; ESMO Guidelines Working Group. Neuroendocrine gastro-entero-pancreatic tumors: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol, 2012, 23(Suppl 7):*vii*124–*vii*130.
[13] Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol, 2008, 26(18):3063–3072.
[14] Lohmann DR, Funk A, Niedermeyer HP, Häupel S, Höfler H. Identification of p53 gene mutations in gastrointestinal and pancreatic carcinoids by nonradioisotopic SSCA. Virchows Arch B Cell Pathol Incl Mol Pathol, 1993, 64(5):293–296.
[15] Ro C, Chai W, Yu VE, Yu R. Pancreatic neuroendocrine tumors: biology, diagnosis, and treatment. Chin J Cancer, 2013, 32(6):312–324.
[16] Lubensky IA, Zhuang Z. Molecular genetic events in gastrointestinal and pancreatic neuroendocrine tumors. Endocr Pathol, 2007, 18(3):156–162.
[17] Couvelard A, Scoazec JY. A TNM classification for digestive endocrine tumors of midgut and hindgut: proposals from the European Neuroendocrine Tumor Society (ENETS). Ann Pathol, 2007, 27(6):426–432.
[18] Lin X, Saad RS, Luckasevic TM, Silverman JF, Liu Y. Diagnostic value of CDX-2 and TTF-1 expressions in separating metastatic neuroendocrine neoplasms of unknown origin. Appl Immunohistochem Mol Morphol, 2007, 15(4):407–414.
[19] Kımıloğlu Şahan E, Erdoğan N, Ulusoy İ, Samet E, Akyıldız İğdem A, Gönüllü D. P53, KI-67, CD117 expression in gastrointestinal and pancreatic neuroendocrine tumours and evaluation of their correlation with clinicopathological and prognostic parameters. Turk J Gastroenterol, 2015, 26(2):104–111.
[20] Warner RR. Enteroendocrine tumors other than carcinoid: a review of clinically significant advances. Gastroenterology, 2005, 128(6):1668–1684.
[21] Alexakis N, Neoptolemos JP. Pancreatic neuroendocrine tumours. Best Pract Res Clin Gastroenterol, 2008, 22(1): 183–205.
[22] Mirancea GV, Moroşanu AM, Carniciu S, Dima S, Bacalbaşa N, Popescu I, Ionescu-Tîrgovişte C, Mirancea N. Relevant infrastructural alterations in a pancreatic neuroendocrine tumor: an insulinoma case. Rom J Morphol Embryol, 2014, 55(2 Suppl): 659–668.
[23] Grant CS. Insulinoma. Best Pract Res Clin Gastroenterol, 2005, 19(5):783–798.
[24] Burns WR, Edil BH. Neuroendocrine pancreatic tumors: guidelines for management and update. Curr Treat Options Oncol, 2012, 13(1):24–34.
[25] Kimura W, Kuroda A, Morioka Y. Clinical pathology of endocrine tumors of the pancreas. Analysis of autopsy cases. Dig Dis Sci, 1991, 36(7):933–942.
[26] Halfdanarson TR, Rubin J, Farnell MB, Grant CS, Petersen GM. Pancreatic endocrine neoplasms: epidemiology and prognosis of pancreatic endocrine tumors. Endocr Relat Cancer, 2008, 15(2):409–427.
[27] Chen M, Van Ness M, Guo Y, Gregg J. Molecular pathology of pancreatic neuroendocrine tumors. J Gastrointest Oncol, 2012, 3(3):182–188.
[28] Massironi S, Sciola V, Peracchi M, Ciafardini C, Spampatti MP, Conte D. Neuroendocrine tumors of the gastro-entero-pancreatic system. World J Gastroenterol, 2008, 14(35):5377–5384.
[29] Ehehalt F, Saeger HD, Schmidt CM, Grützmann R. Neuroendocrine tumors of the pancreas. Oncologist, 2009, 14(5): 456–467.
[30] Raman SP, Hruban RH, Cameron JL, Wolfgang CL, Fishman EK. Pancreatic imaging mimics: Part 2, pancreatic neuroendocrine tumors and their mimics. AJR Am J Roentgenol, 2012, 199(2):309–318.
[31] Halfdanarson TR, Rabe KG, Rubin J, Petersen GM. Pancreatic neuroendocrine tumors (PNETs): incidence, prognosis and recent trend toward improved survival. Ann Oncol, 2008, 19(10):1727–1733.
[32] Capelli P, Martignoni G, Pedica F, Falconi M, Antonello D, Malpeli G, Scarpa A. Endocrine neoplasms of the pancreas: pathologic and genetic features. Arch Pathol Lab Med, 2009, 133(3):350–364.
[33] Johannesen E, Nguyen V. WHO grade 2 neuroendocrine tumor in a 15-year-old male: a case report and literature review. Case Rep Pathol, 2014, 2014:426161.
[34] Modlin IM, Gustafsson BI, Moss SF, Pavel M, Tsolakis AV, Kidd M. Chromogranin A – biological function and clinical utility in neuroendocrine tumor disease. Ann Surg Oncol, 2010, 17(9):2427–2443.
[35] Singh S, Law C. Chromogranin A: a sensitive biomarker for the detection and post-treatment monitoring of gastroenteropancreatic neuroendocrine tumors. Expert Rev Gastroenterol Hepatol, 2012, 6(3):313–334.
[36] Qiao XW, Qiu L, Chen YJ1, Meng CT, Sun Z, Bai CM, Zhao DC, Zhang TP, Zhao YP, Song YL, Wang YH, Chen J, Lu CM. Chromogranin A is a reliable serum diagnostic biomarker for pancreatic neuroendocrine tumors but not for insulinomas. BMC Endocr Disord, 2014, 14:64.

[37] Nehar D, Lombard-Bohas C, Olivieri S, Claustrat B, Chayvialle JA, Penes MC, Sassolas G, Borson-Chazot F. Interest of Chromogranin A for diagnosis and follow-up of endocrine tumours. Clin Endocrinol (Oxf), 2004, 60(5):644–652.
[38] Oberg K, Eriksson B. Endocrine tumours of the pancreas. Best Pract Res Clin Gastroenterol, 2005, 19(5):753–781.
[39] Campana D, Nori F, Piscitelli L, Morselli-Labate AM, Pezzilli R, Corinaldesi R, Tomassetti P. Chromogranin A: is it a useful marker of neuroendocrine tumors? J Clin Oncol, 2007, 25(15): 1967–1973.
[40] Rindi G, Wiedenmann B. Neuroendocrine neoplasms of the gut and pancreas: new insights. Nat Rev Endocrinol, 2012, 8(1):54–64.
[41] Chou WC, Hung YS, Hsu JT, Chen JS, Lu CH, Hwang TL, Rau KM, Yeh KY, Chen TC, Sun CF. Chromogranin A is a reliable biomarker for gastroenteropancreatic neuroendocrine tumors in an Asian population of patients. Neuroendocrinology, 2012, 95(4):344–350.
[42] Öberg K. Diagnostic work-up of gastroenteropancreatic neuroendocrine tumors. Clinics (Sao Paulo), 2012, 67(Suppl 1): 109–112.
[43] Poiană C, Neamţu MC, Avramescu ET, Carşote M, Trifănescu R, Terzea D, Neamţu OM, Ferechide D, Dănciulescu Miulescu R. The poor prognosis factors in G2 neuroendocrine tumor. Rom J Morphol Embryol, 2013, 54(3 Suppl):717–720.
[44] Salyers WJ, Vega KJ, Munoz JC, Trotman BW, Tanev SS. Neuroendocrine tumors of the gastrointestinal tract: Case reports and literature review. World J Gastrointest Oncol, 2014, 6(8):301–310.
[45] Choi YH, Han HS, Lim SN, Lee SY, Koo JH, Lee OJ, Lee KH, Kim ST. Multiple cardiac metastases from a nonfunctioning pancreatic neuroendocrine tumor. Cancer Res Treat, 2013, 45(2):150–154.
[46] Tiemann K, Kosmahl M, Ohlendorf J, Krams M, Klöppel G. Solid pseudopapillary neoplasms of the pancreas are associated with FLI-1 expression, but not with EWS/FLI-1 translocation. Mod Pathol, 2006, 19(11):1409–1413.
[47] Li AF, Tsay SH, Liang WY, Li WY, Chen JY. Clinical significance of p16INK4a and p53 overexpression in endocrine tumors of the gastrointestinal tract. Am J Clin Pathol, 2006, 126(6):856–865.
[48] Rigaud G, Missiaglia E, Moore PS, Zamboni G, Falconi M, Talamini G, Pesci A, Baron A, Lissandrini D, Rindi G, Grigolato P, Pederzoli P, Scarpa A. High resolution allelotype of nonfunctional pancreatic endocrine tumors: identification of two molecular subgroups with clinical implications. Cancer Res, 2001, 61(1):285–292.
[49] La Rosa S, Klersy C, Uccella S, Dainese L, Albarello L, Sonzogni A, Doglioni C, Capella C, Solcia E. Improved histologic and clinicopathologic criteria for prognostic evaluation of pancreatic endocrine tumors. Hum Pathol, 2009, 40(1):30–40.
[50] Klöppel G, Adsay NV. Chronic pancreatitis and the differential diagnosis *versus* pancreatic cancer. Arch Pathol Lab Med, 2009, 133(3):382–387.
[51] Scarpa A, Mantovani W, Capelli P, Beghelli S, Boninsegna L, Bettini R, Panzuto F, Pederzoli P, delle Fave G, Falconi M. Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients. Mod Pathol, 2010, 23(6):824–833.
[52] Klöppel G. Classification and pathology of gastroenteropancreatic neuroendocrine neoplasms. Endocr Relat Cancer, 2011, 18(Suppl 1):S1–S16.
[53] Franchi G, Manzoni MF. Cytological Ki-67 in pancreatic endocrine tumors: a new "must"? Gland Surg, 2014, 3(4): 219–221.
[54] Rorstad O. Prognostic indicators for carcinoid neuroendocrine tumors of the gastrointestinal tract. J Surg Oncol, 2005, 89(3):151–160.
[55] Liu SZ, Zhang F, Chang YX, Ma J, Li X, Li XH, Fan JH, Duan GC, Sun XB. Prognostic impact of cyclin D1, cyclin E and P53 on gastroenteropancreatic neuroendocrine tumours. Asian Pac J Cancer Prev, 2013, 14(1):419–422.
[56] Jain R, Fischer S, Serra S, Chetty R. The use of Cytokeratin 19 (CK19) immunohistochemistry in lesions of the pancreas, gastrointestinal tract, and liver. Appl Immunohistochem Mol Morphol, 2010, 18(1):9–15.
[57] Cheslyn-Curtis S, Sitaram V, Williamson RC. Management of non-functioning neuroendocrine tumours of the pancreas. Br J Surg, 1993, 80(5):625–627.
[58] Holdcroft A. Hormones and the gut. Br J Anaesth, 2000, 85(1):58–68.

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