CHIRURGIA 2003;16:147-50

Non-functioning endocrine tumors of the pancreas
Case report and review of the literature

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The case is reported of a man with non-functional tumor of the pancreas mimicking a pancreatic adenocarcinoma. CT scan showed an enlargement of the pancreatic head (about 3 cm) described as pancreatic carcinoma. Ca19-9 was over 47. The patient underwent surgery in June 2001 and a duodenopacreatectomy with pylorus preservation was performed. The histopathological staging was: non-functioning endocrine pancreatic tumor NSE, cromogranin A and sinaptophysin positive, insulin and glucagon positive in some foci. Ki-67 p.i. less than 3%. Lymph nodes were negative. No adjuvant therapy was performed. Presently the patient is hospitalised for a pneumothorax related to an enphysema progression, but free from neoplastic disease. Pancreatic endocrine neoplasms (PEN) present different onset features with numerous and varied treatment dilemmas. No consensus is actually present in literature as far as prognosis is concerned due to the still unclear definition of PEN malignancies. The histopathological prognostic criteria are: lymph nodes involvement, metastases, vascular invasion, complete resection, size of the primary tumor. Recently, a system has been proposed that has yet to be independently validated. The criteria are: size, mitotic activity, vascular invasion and the tumors are classified as "microadenoma", "macroadenoma", "border line", "low grade carcinoma". Studies on molecular and genetic features of pancreatic endocrine tumors have also been performed. Investigations were carried out on MEN1 genes at chromosome 11q13, Von Hippel Lindau gene, the 4 genes alterated in ductal pancreatic cancer, (p53, K-ras, p16, DPC4), but despite these efforts, little is still known about PENs genetic anomalies. Presently a prognostic histopathological classification may represent a reliable system to approach these neoplasms, but future genetic studies should unable us to better define the prognosis and therapy of these unusual types of cancers.

Key words: Pancreatic neoplasms, diagnosis - Pancreatic neoplasms, physiophatology - Pancreatic neoplasms, genetics.

Non-functioning pancreatic endocrine neoplasms (PENs) are rare epithelial tumors that are usually classified under groups named "endocrine tumors of the duodenopancreatic area" (ETDP), or "pancreatic neuroendocrine tumors" (PETs), or "endocrine or neuroendocrine pancreatic tumors","pancreatic and duodenal neuroendocrine tumors".

Presently no clear definition of these tumors is available both for classification and for prognosis. In a recent publication Hochwald *et al.*[1] reviewed the reports of patients admitted to the Memorial Sloan Kettering Cancer Center during a period of over 10 years and proposed a classification for "pancreatic endocrine neoplasms" (PENs), as "functional" and "non-functional". Furthermore, the better survival for patients with functional tumors compared to patients with non-functional tumors reported by some studies is probably due to the delay in diagnosis in the latter.[2-5] Not all the studies confirm these results.[2-5] Also, no consistent differences in histopathological patterns have been found between the 2 groups.

The case of a man with non-functional tumor of the pancreas mimicking a pancreatic adenocarcinoma and detected at a very early stage gave us the opportunity to review the literature on these topic.

Received April 14, 2003.
Accepted for publication September 24, 2003.

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Case report

A 75-year-old man complaining of a back pain was admitted to our outpatient's service in May 2001. The patient reported to have had that pain for 2 months, with abdominal discomfort and no other significant symptoms. Previous examinations were negative; an ultrasound examination demonstrated only some small cysts in the kidneys, more numerous in the right side. The patient was investigated for upper g.i. pathologies. Tumor markers examination demonstrated a rise in the Ca19.9 (>47). A CT scan showed an enlargement in the

TABLE I.–*Summary of PENs characteristics.*

Functional tumors	Nonfunctional tumors
Criteria	*Lung PENs criteria*
Size	Mitotic rate
Mitotic activity	Necrosis
Vascular invasion	
Groups	*Groups*
Microadenoma	Low grade
Macroadenoma	Intermediate grade
Border line	
Low grade carcinoma	Small, incidental (benign)

pancreatic head (about 3 cm) described as pancreatic carcinoma. The patient underwent surgery in June 2001 and a duodenopacreatectomy with pylorus preservation was performed. Blood sample and fresh frozen specimens were collected as usual for genetic examination, while the surgical specimen was submitted to routine histopathological examination. The histopathological staging was: minimal expression of non-functioning endocrine pancreatic tumor NSE, cromogranin A and sinaptophysin positive, insulin and glucagon positive in some foci. Ki-67 p.i. less than 3%. Lymph nodes were negative. The patient was dismissed from the hospital on day 10 and followed every 3 months with physical examination and tumoral markers evaluation. No adjuvant therapy was performed. A CT scan with manganese was performed after 1 year from surgery. Presently he is hospitalised for enphysema progression and a pneumothorax drainage. No evidence of neoplastic disease is present.

Discussion

Pancreatic endocrine neoplasms (PENs) present different onset features with numerous and varied treatment dilemmas. Broadly these neoplasms can be grouped as "functional" and "non functional" (Table I). Clinical presentation, in the 1st case, is characterized by syndrome caused by hormone hyperincretion, whereas the other is usually induced by the tumor mass.

No consensus is actually present in literature as far as prognosis is concerned [1-8] due to the still unclear definition of PEN malignancies.

Concerning the histopathological definition, the prognostic criteria used are the usual ones (*i.e.* lymph nodes involvement, metastases, vascular invasion). Some authors defined PENs as malignant when lymph nodes are involved or liver metastases are present, but some PENs may recur without metastatic disease or lymphnodal involvement, and others with locally invasive growth, may not.

In a study of 1999, Madeira *et al.*[5] grouped these tumors under the definition "Tumors of the duodenopancreatic area." The authors retrospectively reviewed 82 consecutive patients admitted to the Department of Gastroenterology of Beaujon University Hospital between October 1991 and June 1997. The traditional histological and morphological criteria [9] used for the primary tumor definitions were considered ineffective for prognosis. Furthermore the authors noted also, that other criteria (*i.e.* cell proliferation index) [10] were studied in literature but that as yet none of these were routinely used in the management of patients with this kind of cancer.

As far as non-functioning tumors, the definition concerns tumors with no specific clinical signs induced by hormonal overproduction even if an increase was observed in the plasma level of several hormones and peptides.

Prognostic significant variables at the univariate analysis were: lack of complete resection of the primary tumor, presence of liver metastases, local invasion, primary tumor size >3 cm, non functioning tumors, progression of liver metastases, poor tumoral differentiation. The multivariate analysis showed that liver metastasis was the most significant prognostic factor. When liver metastasis is present, tumor cell differentiation and resection of the primary tumor remains a significant prognostic independent indicator. No histopathological prognostic staging system is so far available for PENs. Recently, a system has been proposed that still needs to be independently validated. This system uses size, mitotic activity and vascular invasion through which tumors are classified as "microadenoma", "macroadenoma", "border line tumor", "low grade carcinoma". On the basis of these criteria, microadenoma and macroadenoma are considered "benign".[8]

In a recent work, Hochwald *et al.*[1] reviewed the topic suggesting that the concept that a PEN removed without recurrence is to be considered as benign, could be challenged. One hundred and thirty-six patients were retrospectively reviewed, who had been hospitalised in the Memorial Sloan-Kettering Cancer Centre in a period of over 10 years. Tumours measuring less than 0.5 cm were defined as "incidentally PENs" or microadenomas and excluded from the study. High grade neuroendocrine carcinomas (neuroendocrine neoplasms with widespread necrosis and >10 mitoses per 10 highpower fields) were also excluded as well as 3 cases of patients with associated MEN1.

In this study the authors assessed the impact on survival of standard clinicopathologic parameters and immunohistochemical staining.

The following parameters were examined specifically: size, presence of lymph nodes or distant metastases, presence of extrapancreatic soft-tissue invasion, presence of vascular invasion, tumor nuclear grade, tumor mitotic rate per 50 HPFs, and presence of tumor necrosis.

Eight-nine patients presented a non functional PEN and the demographics and survival rate of patients with functional PEN were compared. Symptoms of nonfunctional PENs were abdominal pain, palpable mass and jaundice. Approximately 50% of PENs were located in the pancreatic head. Among the 2 groups no difference was found in disease specific survival and disease free survival for curative surgery. For potential curative surgery, the presence of liver metastases in non-functional PENs, tumor necrosis, and MIB-1 value of more than 50 per 10 HPFs worsened the prognosis. The authors also reported that in the group of "microadenoma", considered as benign and "border line", 3 of 18 and 4 of 8 PENs respectively, recurred.

Authors considered difficult to determine the malignant potential of PENs on the basis of histological appearance, and also on the basis of the differences in clinical and pathological criteria used to define their malignancy. From this study emerged that the most important prognostic parameters were mitotic rate and presence of necrosis. Furthermore the difference in survival in metastatic patients suggests that

TABLE II.—*Some recent reports on genetic alteration present in pancreatic endocrine neoplasms, expressely regarding non functioning neoplasms.*

Authors	Alteration examined	Methods	Non functioning pancreatic neoplasm	Results
Chung D.C. *et al.*[11]	VHL Chromosome 3p 25-26	PCR amplification	Yes	VHL does not play a role in sporadic endocrine pancreatic tumor pathogenesis
Bartsch D. *et al.*[12]	DPC4/Smad4	PCR amplification/ single strand conformational variant (SSCP)	Yes	DPC4 alterations is present in 55% of non functional endocrine neoplasm suggesting a role in tumorigenesis
Ebrahimi S.A. *et al.*[13]	Tumor suppressor gene on chromosome 1	PCR amplification	Yes	LOH positive in metastatic patients on chromosome 1
Perren A. *et al.*[14]	PTEN 10q23	PCR amplification	Yes	PTEN is rarely involved in the pathogenesis of EPTs
Rigaud G. *et al.*[15]	Chromosome 6q-11q	PCR amplification	Yes	The allelotype is markedly different from either ductal or acinar tumors of the pancreas as well as functional neoplasm. 11q is common to functional and non functional. 6q is characheristic of NF-PETS
Speel EJ. *et al.*[16]	11p, 3p, 6q, 9q	Comparative genomic hybridization	Yes	Number of gains differed strongly between non functioning and functioning. 9q are present in non functioning and 4 in functioning. 3-6-q losses is related to a malignant behaviour
Barghorn A. *et al.*[17]	6q	Comparative genomic hybridisation/PCR reaction based allelotyping /double target fluorescence *in situ* hybridisation	Yes	On 6q may harbor putative tumor suppressor genes involved in oncogenesis and malignant and metastatic progression of sporadic EPTs
Lubomierski N. *et al.*[18]	P53, CDKN2 on chromosome 9p21,CDKN2A/p16, CDKN2B/p15 and CDKN2D/p14	PCR amplification	Yes	CDKN2A/p16, CDKN2B/p15,CDKN/p14 most frequently alterated in non functional pancreatic endocrine tumors

these 2 groups actually have a different biological behaviour and are not simply different stages in the tumoral evolution, irrespective of hormone hyperincretion.

Recently, studies on molecular and genetic features of pancreatic endocrine tumors have been performed to investigate the behaviour of these tumors (Table II),[11-18] in particular the following genes: MEN1 at chromosome 11q13, Von Hippel Lindau (VHL) as well as the 4 genes frequently alterated in ductal pancreatic cancer, (p53, K-ras, p16, DPC4), but despite these efforts, little is still known about PENs genetic anomalies. In a recent work of Speel *et al.*[16] a study on small tumors was performed using the technique of comparative genomic hybridisation (CGH). Small tumors were defined by the following criteria: dimension of the tumor, presence of angioinvasiveness, number of mitoses and/or number of Ki-67 positive cells. Regarding the endocrine pancreatic tumors, neoplasia less or equal 2 cm harbour the same chromosomal imbalance similar to larger tumor but at a lower frequency. Regarding the nonfunctional tumors, gains of chromosome 4p and 4q were exclusively found in these tumors (40% and 30% of cases, respectively). Nevertheless, the authors themselves suggest caution in interpretating these data. In the same work, the authors also reported an interesting association between metastatic diseases and losses of 1p and gains of 14q.

In another study Rigaud *et al.*[15] studied non functional pancreatic endocrine neoplasm (NF-PETs) by microsatellite analysis of DNA. The study for LOH analysis on chromosome 1,3p, 6q, 9p,11q, 18q was performed both in frozen and in paraffin embedded tissue. The authors found that the allelic deletion involving chromosome 6q is a distinguishing feature of nonfunctioning endocrine pancreatic neoplasms, whereas an alteration on chromosome 11q may be present also in functioning endocrine pancreatic neoplasm. The authors suggested also that other genes can be involved in PENs (*i.e.* ATM gene at 11q22-q23) but further investigations are needed.

Conclusions

In conclusion, in our patient treatment was strongly influenced by a suspect early pancreatic carcinoma; no hyperincretion hormone syndrome was present, and no plasmatic hormone was detectable. We think that the prognostic histopathological classification proposed by Hochwald *et al.* may represent a reliable system to approach these neoplasms, and that genetic studies should unable us to better define the staging system and therapy of these unusual types of cancers.

Riassunto

I tumori endocrini del pancreas non funzionanti. Caso clinico e revisione della letteratura.

I tumori neuroendocrini del pancreas non funzionanti sono una patologia infrequente e, generalmente, sono diagnosticati quando il tumore è di grandi dimensioni e ha già dato metastasi. Riportiamo il caso di un paziente di 75 anni, che all'esordio lamentava disturbi digestivi generici, e dolore a fascia localizzato posteriormente, di tipo subcontinuo che perdurava da circa 2 mesi. Il paziente era stato già studiato per patologie addominali e osteo-articolari; l'unico dato emerso era la presenza di cisti renali bilaterali. L'esame dei marcatori tumorali dimostrava un aumento del Ca 19-9 (>47), la TAC eseguita in seguito, ha evidenziato un aumento volumetrico della testa pancreatica, più evidente a livello dell'uncino (3 cm circa). Il paziente è stato sottoposto a duodenopancreatectomia cefalica con conservazione del piloro. Il pezzo operatorio è stato inviato all'esame istologico, un campione di tessuto a fresco e di sangue sono stati inviati per l'esame delle anomalie genetiche. Il risultato dell'esame istopatologico è stato di tumore endocrino pancreatico a espressione minima, con elementi immunoreattivi per NSE, Cromogranina A e sinaptofisina. Positività focale per insulina e glucagone con indice di proliferazione del Ki-67 <3%. Linfonodi indenni. Il paziente è stato dimesso in 10° giornata e seguito con controlli clinici e umorali ogni 3 mesi. Una TAC con manganese non ha evidenziato ripresa di malattia a 1 anno dall'intervento. Non è stata effettuata terapia adiuvante. L'esordio clinico in questo caso è stato simile a quello del carcinoma pancreatico in fase iniziale con sintomi sfumati di tipo digestivo. Unico dato sospetto il dolore posteriore non riferibile ad altre patologie. L'approccio diagnostico e terapeutico è stato aggressivo e, in questo caso, ha condotto all'eradicazione della neoplasia in fase precoce. Ulteriori studi sulle alterazioni genetiche di queste neoplasie e sulla loro progressione, potranno fornire maggiori prospettive terapeutiche, considerando che, oltre a non essere frequenti, hanno esordio insidioso e arrivano al trattamento in fasi avanzate.

Parole chiave: Pancreas, neoplasie, diagnosi - Pancreas, neoplasie, fisiopatologia - Pancreas, neoplasie, genetica.

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