Original Article

Power Doppler Endoscopic Ultrasound for the Assessment of Pancreatic Neuroendocrine Tumors

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Abstract:

Objective: Pancreatic neuroendocrine tumors (PNET) represent rare, heterogeneous tumors with clinical, imaging and treatment particularities. The aim of this study was to assess the role of power Doppler endoscopic ultrasound (EUS) in the diagnosis and characterization of PNET.

Methods: All consecutive patients with PNET assessed by power Doppler EUS in the Research Centre of Gastroenterology and Hepatology Craiova, Romania, in the past 51 months were included in the study. All EUS examinations were performed initially in gray-scale mode, followed by power Doppler mode examinations, before and after contrast-enhancement. Each recorded EUS movie was further subjected to post-processing using a computer-enhanced dynamic analysis using a special plug-in which permitted assessment of vascularity index (EUS-VI).

Results: Based on the analysis of all consecutive malignant focal pancreatic masses diagnosed in the study period, a total number of 131 consecutive patients were included: 14 patients with pancreatic neuroendocrine tumors and 117 patients with pancreatic adenocarcinoma. The sensitivity of the pre-contrast EUS-VI for the diagnosis of PNET was 71.43%, similar to EUS-FNA. After contrast enhancement, the EUS-VI is also higher in PNET (27.07%) as compared to pancreatic adenocarcinoma where it was significantly lower 9.82% ($P < 0.001$). However, the sensitivity of EUS-VI after contrast enhancement for the diagnosis of PNET was 100%, higher than pre-contrast EUS-VI, with an acceptable specificity (79.49%) and better accuracy (81.68%).

Conclusion: Power Doppler EUS represents a useful method in the initial assessment of PNET. Using evaluation of vascularity through EUS-VI, the differentiation between PNET and pancreatic cancer could be possible, especially in the subgroup of patients where EUS-guided fine needle aspiration is falsely negative.

Keywords: power Doppler; endoscopic ultrasound; pancreatic neuroendocrine tumor

INTRODUCTION

Pancreatic neuroendocrine tumors (PNET) represent rare, heterogeneous tumors with clinical, imaging and treatment particularities. There are only few data in the literature about the role of contrast-enhanced power Doppler endoscopic ultrasound (EUS) in PNET. According to recently published data, contrast-enhanced EUS is a feasible method for the diagnosis of PNET but there are limits regarding differentiating between chronic pancreatitis and PNET, due to the same vascular pattern.[1] European Neuroendocrine Tumor Society (ENETS) established the role of EUS in diagnosis of PNET. EUS-guided fine needle aspiration (FNA) in PNET is mandatory in this set of patients, having an accuracy of about 90% for diagnosis.[2] The immunostaining with chromogranine A and synaptophysine is always necessary.[3-5] The aim of this study was to assess the role of power Doppler EUS in the diagnosis and characterisation of PNET.

MATERIALS AND METHODS

All consecutive patients with PNET assessed by power Doppler EUS in the Research Center of Gastroenterology and Hepatology Craiova, Romania, in the past 51 months (October 2007 - December 2011) were included in the study. EUS- FNA was performed in all suspected focal pancreatic masses, with a minimum of 3 passes performed for both slides and cell blocks (used for cytology and microhistology). It is already known that pancreatic adenocarcinoma is a hypovascular tumor in most of the cases, although hypervascular cases have been described in a minority of patients.[1] On the contrary, neuroendocrine tumors of the
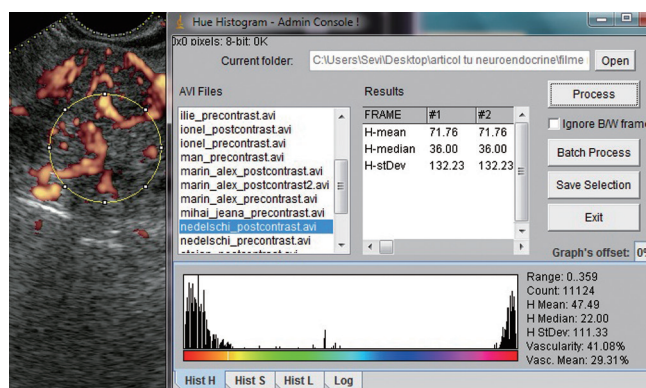
Figure 1. Hypervascular pancreatic neuroendocrine tumor with EUS-VI measurement before and after contrast enhancement (SonoVue).

pancreas are usually well-vascularized. Thus, the diagnosis of PNET was presumed in all hypervascular pancreatic masses and confirmed by a pathological examination of the resection piece or serologic markers of PNET (serum chromogranin A and/or serotonin, urinary 5-hidroxi-indolacetic acid) combined to immunocytochemistry from EUS-FNA aspirates in advanced, not operable cases. Likewise, the diagnosis of pancreatic adenocarcinoma was presumed in all hypovascular pancreatic masses and confirmed by a pathological examination of the resection piece or positive immunocytochemistry obtained from EUS-FNA aspirates in advanced, not operable cases. The differential diagnosis of pancreatic adenocarcinoma with neuroendocrine differentiation was possible only in patients where curative surgical resection allowed a complete immunohistochemical (IHC) examination of the resection material.

Power Doppler EUS procedures were performed with a linear EUS system Pentax EG 3870 UTK coupled with the corresponding ultrasound system Hitachi EUB 8500 or Hitachi Preirus. The following parameters were fixed in power Doppler mode.

All EUS examinations were performed initially in gray-scale mode, followed by power Doppler mode examinations, before and after contrast-enhancement with 2.4 mL of SonoVue (Bracco SpA, Milan, Italy). There are two phases described in contrast-enhanced pancreatic examinations, an early arterial phase (starting from 15 to 30 seconds) and a late venous phase (starting from 30 to 120 seconds).[6] The power Doppler technique was optimized and the gain was set to avoid the appearance of Doppler noise. Thus, after injection of microbubble ultrasound contrast agent, some artefacts usually appear in the arterial phase (blooming or flash artefacts induced by saturation of the signal). Consequently, it is necessary to diminish these artefacts before calculations based on power Doppler analysis. Each recorded EUS movie was further subjected to post-processing using a computer-enhanced dynamic analysis using a public domain Java-based image processing tool (Image J, NIH, Bethesda, Maryland,
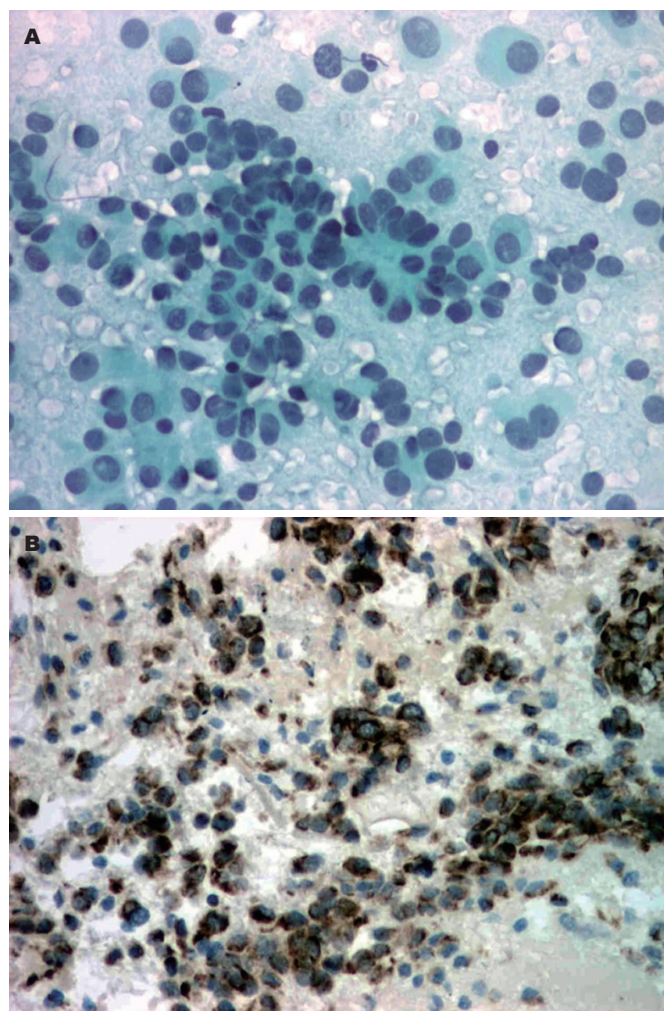

Figure 2. Cytology exam of pancreatic neuroendocrine tumor. A: Small regular cells with pseudoglandular arrangement; B: Positive immunocytochemistry for chromogranine A staining.

USA) with a special vascularity plug-in developed by the IT Department of the University of Medicine and Pharmacy, Craiova. The plug-in was used to calculate the percent of color pixels of each frame of the movie. Thus, the EUS vascularity index (EUS-VI) was calculated before and after contrast-enhancement, as a percent of color pixels in every frame of the movies, with an average calculated over a 10-second movie (Fig. 1).

For cytological examinations, Giemsa and Papanicolau stainings were used in the first step (Fig. 2A). Then, an immunoassay with synaptophysine and/or chromogranine A was performed in all patients to demonstrate the neuroendocrine origin, based on cell blocks obtained through EUS-FNA (Fig. 2B). The staining for the proliferation marker Ki-67 was realized only in patients who underwent surgery, based on histopathological examination of resection pieces.

The sensitivity, specificity, positive predictive value

(PPV), negative predictive value (NPV), and accuracy for the diagnosis of PNET were calculated for both power Doppler EUS, as well as for EUS-FNA. Analyzing a 2 × 2 contingency table was used for statistical analysis, while P value was obtained using Fisher's test. A P value below 0.05 was considered statistically significant.

RESULTS

Based on the analysis of all consecutive malignant focal pancreatic masses diagnosed in the study period, a total number of 131 consecutive patients were included: 14 patients with pancreatic neuroendocrine tumors and 117 patients with pancreatic adenocarcinoma. PNET represented 7.41% of 189 malignant pancreatic solid masses assessed by EUS-FNA in the past 51 months in our center.

From the 14 patients with PNET, 6 were females and 8 males. The age of patients with PNET was 54 ± 14.76 years while the age of patients with pancreatic adenocarcinoma was 62.40 ± 11.24 years, which was significantly higher ($P = 0.0119$).

EUS and Clinico–pathological Characteristics of PNET

The size of the tumors was 3 ± 1.46 cm. The main localization was the head (35.71%) and body of the pancreas (25.7%). There were 2 (14.28%) tumors localized at the uncinate process and 3 (21.43%) tumors on the tail. Only 2 tumors were of secretory type (insulinomas) while the majority of them were of non-secretory type (85.71%).

The majority of PNET were well vascularized (Fig. 3A); only 2 tumors were hypo-vascularized as compared to normal pancreatic parenchyma (Fig. 3B). Thus, qualitative assessment of vascularity in a tumor was used in statistical analysis. Sensitivity of the method was 85.71%, specificity 93.04%, and accuracy 90.84%, respectively. The EUS-VI was calculated before and after contrast (SonoVue) enhancement. Thus, the EUS-VI in PNET before contrast enhancement was 8.06%, while in pancreatic adenocarcinoma the VI was significantly lower 2.72% ($P < 0.001$). The sensitivity of the pre-contrast EUS-VI for the diagnosis of PNET was 71.43%, similar to EUS-FNA. After contrast enhancement, the EUS-VI was also higher in PNET (27.07%) as compared to pancreatic adenocarcinoma where it was significantly lower (9.82%, $P < 0.001$). However, the sensitivity of EUS-VI after contrast enhancement for the diagnosis of PNET was 100%, higher than pre-contrast EUS-VI, with an acceptable specificity (79.49%) and better accuracy (81.68%). EUS-VI did not correlate to age, gender, and TNM stage in neuroendocrine tumors of pancreas (Tab. 1), and thus it should be considered in further studies as an independent predictor of the prognosis.

Overall, addition of FNA to conventional EUS did not increase significantly the sensitivity (71.43%) as compared to pre-contrast EUS-VI, due to sampling errors in small tumors. The specificity of EUS-FNA in the diagnosis of PNET was however 100% and accuracy 96.95%, with a PPV of 100%
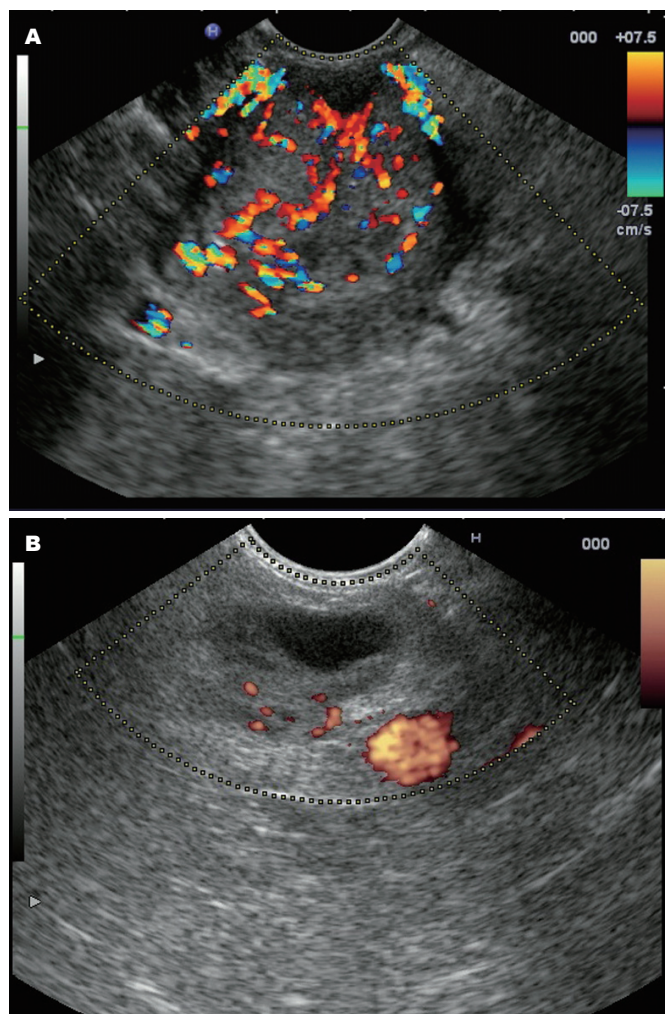

Figure 3. Power Doppler EUS assessment of pancreatic neuroendocrine tumor. A: Hypervascular malignant PNET; B: Hypovascular PNET (insulinoma).

and a NPV of 96.69% (Tab. 2). Only one minor complication was recorded after EUS-FNA consisting in a retroperitoneal haemorrhage which stopped spontaneously.

The main aspect on cytological smears was the presence of small, round cells with pseudo-glandular arrangement (Fig. 2B). The Ki-67 marker was stained in only 8 (57.14%) patients, which were operated on. Thus, this marker was not included in the statistical analysis.

Staging of PNET

The majority of patients were diagnosed in T1-T2 stages (57.12%), but there were a large number of patients in advanced T stage (42.88% in T4 stage). Liver metastases were found in 3 out of 14 cases (21.43%), while loco-regional or distant malignant lymph nodes were found in 7 cases (50%). TNM staging according to American Joint Cancer Committee (AJCC) revealed that 50% of patients were in the first and second stages (stage 1a: 21.43%; stage 1b: 21.43%, 2b: 7.14%), but there were 7 patients in advanced stages (stage 3: 28.57%;

Table 1. Vascularity index and clinico-pathological parameters in pancreatic neuroendocrine tumors

Clinico-pathologic features	Pre-contrast vascularity index			Post-contrast vascularity index		
	< 8.06	>8.06	*P* value	< 27.07	>27.07	*P* value
Age						
<54 years	3	4	1.0000	4	2	0.5921
>54 years	3	4		2	5	
Gender						
Males	6	2	0.1026	4	4	0.6270
Females	1	5		2	4	
T grade						
T1-T2	5	3	1.0000	4	4	0.6270
T3-T4	3	3		2	4	
N grade						
N0	3	3	1.0000	3	3	1.0000
N1	4	4		3	5	
M grade						
M0	4	6	0.1923	4	7	0.5385
M1	3	0		2	1	
TNM stage						
Stage 1-2	4	3	1.0000	3	4	1.0000
Stage 3-4	3	4		3	4	

stage 4: 21.43%).

DISCUSSION

The main type of PNET was the non-functioning one, similar to recently published data.[2] The medium age of patients with PNET was lower than that of patients with pancreatic adenocarcinoma, but higher than that with chronic pancreatitis. In detection of PNET, EUS seems to be the better imaging diagnostic tool, having an accuracy of 93%, even if it is compared to multidetector computed tomography (CT).[8,9]

Although it is well known that PNET are hypervascular tumors, the quantification of vascularization using power Doppler parameters is not yet sufficiently studied. Our paper comes to offer a feasibility study for establishing the role of EUS-VI in the assessment and diagnosis of PNET. This parameter has already been studied in pancreatic adenocarcinoma where it offered a good sensitivity, specificity and accuracy of 75.8%, 95.2%, and 83.3%, respectively, while it could also be used in combination with real-time elastography.[10] Evaluation of vascularity through EUS-

VI might thus be a good tool for differentiation between PNET and pancreatic adenocarcinoma after microbubble contrast enhancement (SonoVue). This parameter cannot be used for the differentiation between PNET and chronic pancreatitis, both masses being hypervascular in most of the cases. Furthermore, the advent of low mechanical index (MI) techniques will further allow the evaluation of microvascularity and perfusion in PNET, thus allowing a sensitive method for precise diagnosis, accurate staging, and also follow-up during specific treatment with somatostatin analogs or antiangiogenic drugs.

In pancreatic cancer, the sensitivity of EUS-FNA for the diagnosis of malignancy was around 85%, while the specificity was 98%, according to a recently published meta-analysis.[11] In the setting of patients with PNET, we obtained a lower sensitivity of EUS-FNA (71.43%) for the diagnosis of PNET, but a better accuracy (96.95%). In the literature, the data were also discordant, and EUS-FNA accuracy in PNET varied between 46% and 90.1%, probably due to the small number of patients and lack of standardization of EUS-FNA sampling and evaluation techniques.[2,7,8] The lower accuracy in some studies might be due to sampling error or

Table 2. The role of EUS tools in diagnosis of pancreatic neuroendocrine tumors

	Sensitivity	Specificity	Accuracy	NPV	PPV
Qualitative vascularity	85.71	93.04	90.84	98.16	60
EUS pre-contrast VI	71.43	77.78	77.10	95.79	27.78
EUS post-contrast VI	100	79.49	81.68	100	36.84
EUS-FNA	71.43	100	96.95	96.69	100

EUS: endoscopic ultrasound; EUS-FNA: EUS-guided fine needle aspiration; NPV: negative predictive value; PPV: positive predictive value.

hemorrhagic samples in these hypervascular tumors, as well as lack of routine performance of immunocytochemistry.[12]

The cytological immunostaining offers an accurate diagnosis of PNET. In our study, the main cytological aspect was the presence of small round cells in pseudo-glandular/ rosette arrangement, similar to other aspects from the literature. Thus, according to a study focused on cytology in PNET, the most helpful cytological feature that facilitated the cytological diagnosis of PNET was a monotonous, poorly cohesive population of small cells with plasmacytoid morphology.[13] In the diagnosis of PNET, the proliferation marker Ki-67 was mandatory.[5] In our study, only 8 patients (57.14%) had Ki-67 assessment. Usually, this marker is assessed by immunohistochemistry in surgical samples. According to recent data, it seemed to be feasible to calculate the proliferation index as a percentage using Ki-67 staining on immunocytochemistry.[14]

Our study has several limitations, which included mainly the small number of patients. Also, the use of newer low-MI techniques during contrast-enhanced harmonic EUS might further improve the quantitative evaluation of vascularity in PNET through time-intensity curve (TIC) analysis.[15] This might offer the opportunity of follow-up during treatment of advanced inoperable cases with either somatostatin analogues or antiangiogenic drugs, because Response Evaluation Criteria in Solid Tumors (RECIST) were not enough for the evaluation of hypervascular tumors during antiangiogenic treatment.[16]

In conclusion, power Doppler EUS represents a useful method in the initial assessment of PNET. By evaluation of vascularity through EUS-VI, the differentiation between PNET and pancreatic cancer could be possible, especially in the subgroup of patients where EUS-FNA is falsely negative. This does not preclude the use of EUS-FNA with immunocytochemistry, which has an acceptable sensitivity for the diagnosis of PNET and a very high specificity and accuracy.

ACKNOWLEDGEMENT

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