Endoscopic ultrasound-guided fine needle aspiration in the evaluation of pancreatic neuroendocrine neoplasms

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Neuroendocrine tumors (NETs) of the pancreas are relatively uncommon tumors that account for 1-2% of all pancreatic neoplasms.[1] The peak incidence is from ages 30-60 years, although cases have been described at all ages.[2,3] These tumors originate predominantly from the pancreatic islets of langerhans and are thus known as islet cell tumors,[3,4] or can arise from the multipotent ductular stem cells.[4] The NETs are commonly associated with clinical syndromes directly related to a hormone secreted by the tumor. These functional tumors are classified based on the hormones they produce and the associated endocrine syndrome. The precise localization of NETs is of major importance because surgical resection is the only curative treatment. Endoscopic ultrasonography (EUS) uses the technology of endoscopy to introduce high-frequency ultrasound probes in the upper, or lower part of the gastrointestinal (GI) tract to visualize its wall and adjacent structures. The EUS is proven to be a highly accurate clinical diagnostic tool for the diagnosis, staging, and optimal management of pancreatic neoplasms, including NETs,[5] which allows the detection of lesions that measure less than one cm. The EUS is also used to evaluate the extent of lesions in the adjacent lymph nodes. The EUS-guided fine-needle aspiration (FNA) provides physicians with the cytologic diagnosis of such lesions with a sensitivity, and specificity approaching 98% and 100%. The EUS-guided FNA is also minimally invasive; with a low complication rate. The EUS-FNA does not require general anesthesia, or hospitalization. The objective of this report was to reaffirm the diagnostic importance of EUS-FNA in the evaluation of pancreatic NETs, and describe the cytopathologic and immunocytochemical features of NETs obtained by EUS-FNA.

Six patients with pancreatic NETs were diagnosed by EUS-FNA cytology between May 2007 and June 2010 at the King Khalid University Hospital, King Saud University, Riyadh, Kingdom of Saudi Arabia. The patient's charts were reviewed, and clinical information obtained. All patients were referred for EUS-guided FNA examination for suspicion of pancreatic masses/nodules. All included cases had confirmative diagnosis either by cytomorphologic and immunocytochemical findings, or by subsequent surgical excision. Cases with no adequate cytomorphologic material/features or confirmative surgical samples were excluded. All cytology specimens, and cell block procedures were performed in the endoscopy suite. The aspirated samples were assessed immediately by an on-site cytotechnologist in all cases. The aspirated material was smeared onto slides, and smear preparation was followed by either air drying for Diff-Quick staining, or immediate fixation in 95% ethanol for subsequent Papanicolaou staining methods. Additional aspirated material was obtained for cell block preparation, fixed in formalin, embedded in paraffin, and processed for routine histologic examination using standard techniques. On average, 3, or 4 passes finally were performed to obtain diagnostic material. Immunocytochemical stains were performed on cell block preparations to determine neuroendocrine differentiation. For this purpose, 5-mm sections were cut, deparaffinized, and mounted on pre coated slides. The following antibodies were used for immunocytochemistry (ICC) studies, all from Novocastra, Newcastle, UK: synaptophysin, chromogran, CD56, and cytokeratin (CK). Occasionally, for differential diagnosis, the following antibodies were obtained, CK7, CK20, Progesteron, B-catenin, E-cadherin, CD10, and Vimentin.

Table 1 - Clinical, endoscopic ultrasound, and cytological features of the patients with pancreatic neuroendocrine tumors.

Patient	Age/gender	Size/location	Symptoms	Cytomorphology	Immunocytochemistry
1	46/F	7x6 cm/B	Pain, vomiting	Suspicious for NET	Not carried out
2	45/F	3x3 cm/H	Pain, diarrhea, constipation	Suspicious for NET	Not carried out, on surgery positive for CD56, syn, chrom
3	70/M	1.7x1.5 cm/H	Pain	NET	Positive for CD56, syn, chrom
4	56/F	6x5 cm/B	Pain, vomiting	NET	Positive for CD56, syn, chrom
5	34/M	6x5 cm/B+H	Pain	NET	Positive for CD56, syn, chrom
6	69/M	6.6x4.2 cm/H	Pain	NET	Positive for CD56, syn, chrom

EUS - endoscopic ultrasound, FNA - fine needle aspiration, H - head of pancreas, B - body of pancreas, syn - synaptophysin, chrom - chromogranin, NET - neuroendocrine tumor

Cytopathologic characteristics that were studied in the smears were degree of samples cellularity, single cell distribution versus clustering, nuclear size, shape, location, and nuclear membrane abnormality, nucleoli size, number, and size, chromatin distribution, cytoplasmic features, necrosis, and mitotic activity. Cytopathologic diagnoses subsequently were correlated with the final histologic diagnoses in 2 patients, who underwent surgical resection. The clinical data, and EUS findings are summarized in **Table 1**. Three patients were men, and 3 patients were women. The mean age of patients was 56.3 years (range, 34-70 years). Three tumors were located in the head of the pancreas, 2 tumors were located in the body, and one tumor was located in both the body, and the head of the pancreas. On EUS, the tumors ranged in size from 1.7-7 cm (mean 5.06 cm). The EUS revealed a solid, hypoechoic mass in 5 patients. In one case there was a cystic component in addition to the solid mass. Peripancreatic/abdominal lymph node enlargement was seen in 4 patients. Hepatic involvement was seen in one patient. One patient was previously diagnosed with 2 primary cancers, one was renal cell carcinoma in the left kidney, and the other was recto-sigmoid adenocarcinoma, in addition to the newly diagnosed pancreatic NET. Surgery was performed in 2 patients, and the diagnosis was confirmed in both as NET. Immunocytochemistry studies were obtained in 4 cases. In 2 cases, the cell block material was not adequate to perform it. The FNA smears were highly cellular in all cases. The aspirates revealed predominantly single cell population and often contained loosely cohesive groups, and rosette-like formations. Cells were small to medium in size with moderate amount of pale to eosinophilic cytoplasm and remarkably uniform, monotonous, small to medium-sized, round to oval, and frequently peripherally located (plasmacytoid appearance) nuclei with finely distributed, "salt-and-pepper" chromatin. Nuleolei were inconspicuous, or small. The background frequently was bloody. Mitotic figures, and necrotic cell debris were noted rarely. Four tumors were diagnosed as NETs according to the cytomorphologic features and were further confirmed by positive immunostaining for neuroendocrine markers. Two tumors were diagnosed as suspicious of NETs; ICC was not available in both because of the lack of sufficient cell block material. The histologic diagnosis was available in 2 patients, who underwent surgery, both showed the characteristic features of NETs.

The benign, normal acinar epithelium seen in some smears was arranged in small acini. The normal epithelium of the pancreatic ducts, also seen frequently in smears and was composed of columnar, mucinous epithelium in flat sheets in which the nuclei were located centrally with absence, or minimal pleomorphism. The GI epithelium related to the stomach, and the duodenum consisted of columnar, mucin-containing, goblet cells

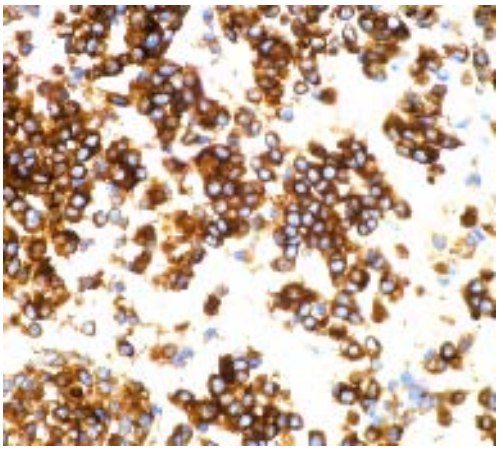

Figure 1 - Cell block preparation. The neuroendocrine differentiation is confirmed by strongly positive immunostaining for synaptophysin (40X).

in large honeycombed sheets with a luminal border. The ICC staining of cell block preparation was performed in 4 cases, in all the neuroendocrine markers including synaptophysin (**Figure 1**), chromogranin A, and CD56 were diffusely to focally positive. From time to time other immunocytochemical stains were used (CK A1/A3, CK7, CK20, Ecadherin, B-catenin, Vimentin, CD10 and Progesterone) to exclude other possibilities such as pancreatic solid papillary neoplasm, ductal pancreatic adenocarcinoma, and other metastatic cancers.

Despite the large number of publications regarding its diagnostic and staging capabilities, EUS-FNA has been limited to a small number of academic centers worldwide. The EUS-FNA cytology interpretation requires an extraordinarily high experience, and high level of cooperation and trust between the gastroenterologist, and the cytopathologist. The EUS-FNA of pancreatic neuroendocrine neoplasms presents real diagnostic challenges to cytopathologists. There are a few unique features associated with the EUS-FNA technique. The most important one was the presence of variable components of normal duodenal, or gastric epithelial cells, and normal pancreatic acini and ducts. Distinguishing normal from abnormal cellular elements is not always easy, as the presence of abundant normal cellular elements may either obscure the neoplastic cells, or more seriously mimic them. Cell block preparation can be used not only to confirm the neuroendocrine origin of the neoplastic cells and to perform ancillary studies when necessary, but it might contain the only diagnostic material to confirm NETs. The current study highlighted the cytomorphologic findings of NETs of the pancreas from 6 patients. Only a few large series, similar to our report, with sample size ranging from 6-20 reported from multiple institutions have described the cytomorphologic, and immunocytochemical features of NETs of the pancreas.[5] Our study demonstrated that

the most helpful cytomorphologic features used for the cytopathologic diagnosis of NETs were uniform, monotonous, poorly cohesive population of small to medium size cells with plasmacytoid morphology. However, many times, cytomorphologic samples obtained from pancreatic NETs tend to be bloody, with normal GI elements. In general, the reported accuracy for pancreatic NETs was lower in comparison with that reported for pancreatic ductal adenocarcinoma. The diagnostic accuracy for NETs was fortified when combined with the ICC performed on cell block. The ICC stains play a crucial role in confirming the neuroendocrine origin of tumor cells. In our study, whenever we had enough material on the cell block preparation, neoplastic cells were always positive for neuroendocrine markers including chromogranin A, synaptophysin, and CD56. The main differential diagnosis of NETs includes ductal adenocarcinoma, and solid pseudopapillary tumor. Ductal pancreatic adenocarcinomas are typically well to moderately differentiated, show gland formation and have characteristic cytomorphologic changes that are well delineated, and approved previously in many reports. Overall, adenocarcinoma reveals clusters and single forms of atypical columnar epithelial cells with remarkable pleomorphism, and nuclear enlargement. Immunocytochemistry can confirm the neuroendocrine neoplasm diagnosis. Solid pseudopapillary neoplasms of the pancreas usually occur in middle aged females, and cytologically shows cellular smears with papillary formation, bland nuclei with focal grooving, and moderate amount of eosinophilic cytoplasm. In addition, the ICC can help remarkably by using specific set of markers in difficult cases to reach the correct diagnosis.

In conclusion, NETs of the pancreas are rare and the current study, though limited by the low number of cases that were included, supports the role of EUS-guided FNA in diagnosing pancreatic NETs. By typical cytomorphologic findings, along with the use of ancillary ICC stains, the cytopathologist confidently could reach an accurate diagnosis in most cases.

Received 28th August 2010. Accepted 27th September 2010.

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