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Role of Guided – Fine Needle Biopsy of the Pancreatic Lesion

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1. Introduction

Pancreatic cancer (PC) is the fifth leading cause of cancer death in the United States, with 28,000 to 30,000 number of deaths annually (American Cancer Society, 2002).

Survival in patients with untreated pc is very poor, the one year survival rate is 19% and the 5- year survival rate is 4% for all stages combined (American Cancer Society, 2002).

It must be emphasized that the majority of patients with pancreatic cancer are diagnosed in the metastatic phase; however when complete surgical resection with margin negative and node negative is possible, it offers the best opportunity for long survival or even cure, with 5-year survival approaching 40% when performed at specialized center. (Sohn et al, 2000).

Epithelial neoplasia of pancreas can be divided into those with predominantly exocrine differentiation and those with endocrine differentiation.

Neoplasia of exocrine differentiation can be further subdivided into solid and cystic tumors; the majority of malignancies of the pancreas are solid infiltrating ductal adenocarcinomas.

2. Histology

About 80% of pancreatic malignancies are ductal adenocarcinomas, of which approximately 70% occur in the head of the pancreas.

A variety of uncommon types of pancreatic carcinoma have been described, including acinar, adenosquamous, anaplastic, papillary, mucinous and microadenocarcinomas, each of which composes less than 5% of the total. All of these have similarly poor prognoses and are treated in a similar fashion. Also uncommon are mucinous cystic neoplasms (cystadenoma/cystadenocarcinoma) of the pancreas, which occur most frequently in the middle-aged women, and these are typically located in the tail of the pancreas.

Clinical behavior can be difficult to predict pathologically, leading some to conclude that all mucinous cystic neoplasms of pancreas have malignant potential.

Other rare neoplasms include pancreatoblastomas, most of which occur in children, primary lymphoma of the pancreas and metastasis.

3. Diagnosis of pancreatic neoplasia

Currently, imaging modalities for detection of pancreatic masses include ultrasonography (US), computed tomography (CT) scan, magnetic resonance imaging (MRI)/ magnetic resonance cholangiopancreatography, endoscopic ultrasonography (EUS).

In clinical practice differential diagnosis of pancreatic masses is frequently a clinical challenge; often therapeutic decision in this context is mainly based on the ability to perform a diagnosis of malignancy or to exclude malignancy (Tamm & Charnsangavej, 2001).

It is well known that ductal adenocarcinoma is the most frequent cause of pancreatic mass, however other neoplasms such as lymphoma, metastasis, cystic tumors or benign conditions as chronic pancreatitis with different prognosis and treatment options can arise within the pancreas (Iglesias et al., 2010).

A pathologic diagnosis becomes therefore relevant for an adequate therapeutic strategy (Cohen et al., 2000).

At least 80% of the patients with suspected pancreatic cancer, have unresectable disease at diagnosis because of locoregional involvement or distant metastases, and it has been reported that only 7% of the patients have a tumour that is confined within the pancreas (National Cancer Institute, 2007).

Patients with suspected pancreatic cancer and imaging studies suggesting resectable tumour should undergo directly to surgery since no histologic diagnosis confirmation is required prior to surgical exploration unless neoadjuvant therapy is indicated (Zamboni et al., 2010).

As a matter of fact preoperative cytohistological diagnosis may risk dissemination of cancer cells, or developing complications (bleeding, pancreatitis, pancreatic leak) that can delay surgery and increase costs.

On the other hand a negative biopsy results in a patient with a high suspicion of cancer neoplasm that is not of help, due to a high possibility of a false negative result (Tillou et al., 1996).

Patients with metastatic or locally advanced but unresectable disease at imaging studies should undergo biopsy prior chemotherapy or radiation since a cytohistological diagnosis is recommended before initiating a cytostatic therapy coherently with the National Comprehensive Cancer Network (NCCN) guidelines for suspected pancreatic cancer. (Hartwig et al, 2009; Itani et al., 1997) Biopsies allow a cytohistological diagnosis and can differentiate pancreatic cancer between primary pancreatic lymphoma (Arcari et al., 2005), metastasis or benign focal lesion such as focal pancreatitis.

3.1 Primary pancreatic lymphoma and pancreatic metastasis

Primary pancreatic lymphoma (PPL) is a very rare disease, representing fewer than 2% of extra-nodal malignant lymphoma and 0,5% of all pancreatic masses (Arcari et al., 2005).

Fewer than 150 cases of PPL have been reported in the literature in English. Imaging techniques such as US and CT scan can suggest a diagnosis of PPL but a cyto-hystological examination is mandatory for diagnosis and treatment planning of patients with suspicious PPL. Our group reported five cases of PPL and reviewing the literature it was concluded

that 1) imaging techniques can suggest the suspicion of PPL, however are unable to distinguish PPL from pancreatic adenocarcinoma, 2) histological diagnosis can be easily obtained by percutaneous US-guided tissue core biopsy 3) surgery can be avoided both for diagnosis and therapy, but the treatment of choice of PPL may only be evaluated on a larger series of patients (Arcari et al., 2005).

Metastases to the pancreas are rare; in a survey of 4,955 autopsies (Adsay et al., 2004) a rate of metastasis to the pancreas of 3,83% was described and a significantly different distribution of metastatic neoplasms, with lung and gastrointestinal tumors comprising by far the largest proportion.

In a retrospective review of 1,172 pancreatic endoscopic ultrasound-guided fine-needle aspiration biopsy, 25 cases (2,1%) had a confirmed diagnosis of pancreatic metastasis.

This included 12 cases of renal cell carcinoma, 3 (12%) melanomas, 3 (12%) small cell carcinomas and 7 (28%) other malignancies.

In these metastatic tumors involving the pancreas 20 (80%) of the lesions were solitary.

Four cases (16%) had no prior history of malignancy; the average time of diagnosis of pancreatic metastasis was 5.3 years.

Immunohistochemistry and special stains were performed in 22 (88%) and 9 (36%) cases respectively (Gilbert et al., 2011).

4. Staging of pancreatic cancer (table 1)

Staging procedures include US, CT scanning, MRI, and EUS. A diagnostic laparoscopy may also be performed to detect peritoneal disease that is not visible radiologically. Regardless of these studies, an accurate histologic diagnosis is necessary to distinguish benign disease from carcinoma, islet cell tumors, and retroperitoneal lymphomas, because of the major therapeutic and prognostic differences among these disease entities. Criteria for surgical resection include absence of metastatic disease and absence of invasion of prominent local blood vessels.

5. Guide for pancreatic biopsy

Intraoperative needle biopsy of the pancreas has been performed with the fine-needle aspiration biopsy (FNAB) technique since the 1960's (Moossa & Altorki, 1983) and with the core tissue biopsy technique since 1970's (Ingram et al., 1978) subsequently ultrasound, computed tomography and endoscopic ultrasound became available to evaluate and characterize pancreatic masses and above all to guide a needle for the biopsy, avoiding the costs, morbidity and mortality of a major surgical procedure performed only to obtain a tissue sample for a cytohistological diagnosis (Civardi et al., 1986; Turner et al., 2010).

For many years percutaneous US and /or CT guided biopsy was routinely performed in situations in which a pancreatic biopsy was necessary, in 2002 the American Joint Committee on cancer has selected endoscopic ultrasound guided FNAB as "the procedure of choice" if available (Greene et al., 2002).

However, as recently reported, local expertise in and the availability of EUS and interventional radiology may determine the first procedure selected for a cytohistological diagnosis for a pancreatic mass (Zamboni et al., 2010).

Prymary Tumor (T)			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
Tis	In situ carcinoma		
T1	Tumor limited to the pancreas 2 cm or less in greatest dimension		
T2	Tumor limited to the pancreas more than 2 cm in greatest dimension		
T3	Tumor extends beyond the pancreas but without involvement of celiac axis or the superior mesenteric artery		
T4	Tumor involves the celiac axis or the superior mesenteric artery (unresectable primary tumor)		
Regional Lymph Nodes (N)			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Regional lymph node metastasis		
Distant Metastasis (M)			
MX	Distant metastasis cannot be assessed		
M0	No distant metastasis		
M1	Distant metastasis		
Stage grouping			
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T1-3	N1	M0
Stage III	T4	Any N	M0
Stage IV	Any T	Any N	M1

Table 1. Staging of pancreatic carcinoma

5.1 Methods of percutaneous guided biopsy

Patient preparation before any type of invasive procedure includes ruling out coagulation disorders with laboratory tests and obtaining written informed consent for the biopsy. Local anesthesia (lidocaine) is not routinely performed. (Zamboni et al., 2010; Civardi et al., 1986).

5.2 Percutaneous ultrasound

In the past for performing abdominal US-guided FNAB the “free-hand” technique was utilized (Civardi et al., 1986; Bret et al., 1982; Livraghi, 1984) subsequently puncturing probe became available and two types of probes are commonly used for interventional procedures: probes with lateral support and probes with noncontinuous crystals and central support; the former allow only oblique needle tracks, whereas the latter allow both vertical and oblique tracks.

Prior to perform the biopsy, a pancreatic lesion can be studied with conventional US, Doppler US, and CT, to evaluate the content of the lesion and to select the best route for biopsy, avoiding vessels and pleura.

In clinical practice when liver metastases are present in patients with suspected pancreatic cancer, the biopsy can be done in the liver metastasis, if safer for the patient and easier for the physician.

5.3 Computed tomography

Computed tomography allows optimal visualization of the lesion and is superior to US in large fat patients, however radiation dose and the procedure length are the major limits of CT-guided pancreatic biopsy.

CT fluoroscopy can reduce procedure length because it allows a fast reconstruction of images, with a continuous update and the possibility of controlling acquisition and visualizing images in the room while performing the examination.

In addition CT fluoroscopy allows visualizing the needle track from the entry point to the target, allowing faster and more efficient procedure (Zamboni et al., 2010).

5.4 Endoscopic ultrasound

Over the past decade, EUS has proven to be one of the most significant advantages in gastrointestinal endoscopy (Turner et al., 2010; Erickson, 2004). Since its introduction, EUS has offered improved accessibility to small pancreatic lesions, and its usefulness as a diagnostic tool has greatly changed the therapeutic approach to pancreatic masses.

Since it was first reported in (Chang et al, 1994), EUS-guided FNAB of the pancreas has become a popular technique for the diagnosis and staging of cystic and solid lesions of the pancreas because it is relatively safe and accurate (Carrara et al., 2010;).

Thus, this diagnostic modality has become important in the management of patients with symptomatic or incidentally discovered pancreatic masses.

5.4.1 US, CT, or EUS for guide pancreatic FNAB

The relative diagnostic accuracy, safety and cost of US and CT-guided FNAB favor their use over EUS-FNAB for the diagnosis of unresectable pancreatic tumors. (Zamboni et al., 2010; Levy, 2006).

A randomized controlled trial EUS-FNAB and US/CT-FNAB failed to observe any statistically significant difference between the endoscopic and percutaneous approach in the diagnosis of pancreatic malignancy (Horwhat et al., 2006).

Several authors support the use of EUS-FNAB over percutaneous approach because of the lower risk of seeding (Gilbert et al, 2001; Turner et al, 2010). In a review of 1406 cases with advanced pancreatic cancer who underwent nonsurgical biopsy (percutaneous-guided or EUS-guided sampling) were compared with cases who did not undergo biopsy, without observing any difference in overall median survival, so it was concluded that the risk of

seeding is remote. (Hernandez et al., 2009). It was reported that the risk of seeding can be related to the number of needle passes: more number of needle passes more risk of seeding (Civardi et al., 1986; Fornari et al., 1989).

It must be emphasized that one-site cytopathological evaluation can improve the diagnostic yield of guided FNAB and can reduce the number of needle passes (Garcia et al., 2011).

A review of 182 patients undergoing EUS-guided FNAB of solid pancreatic lesions over a 2 years study period was reported (Garcia et al., 2011). Sample were either evaluated on site by a cytopathologist or processed by the endoscopist and sent to the pathology department for evaluation.

Diagnostic accuracy for malignancy, number of needle passes, adequate – specimen collection rate, cytological diagnosis, and final diagnosis and complications rate according to the presence or absence of on-site cytopathologist were evaluated.

A significantly higher number of needle passes was performed when an on-site cytopathologist was not available (3.5 ± 1.0 vs 2.0 ± 0.7 ; $p < 0.001$). The presence of an on-site cytopathologist was associated with a significant lower number of inadequate samples (1.0 vs 12.6%; $p = 0.002$) and a significantly higher diagnostic sensitivity (96.2 vs 78.2%; $p = 0.002$), and overall accuracy (96.8 vs 86.2%; $p = 0.013$) for malignancy (Garcia et al., 2011).

Already, in 1988 our group reported the value of rapid staining and assessment of percutaneous ultrasound-guided fine needle aspiration biopsy in a series of 160 patients. (Civardi et al., 1988) The total series of FNAB had a sensitivity of 95.6%, a specificity of 100% and an overall accuracy of 97.3%.

The cumulative accuracy after each pass was calculated: a significant increase in diagnostic accuracy was found only after the second pass, the third and the fourth passes gave little further improvement. These results indicate that a rapid evaluation of the aspirated material during US-guided FNAB can reduce the number of punctures needed per case resulting in less discomfort and, probably a reduced likelihood of complications for the patient.

It must be emphasized that in this study the same physicians that performed the US-FNAB performed also the rapid staining evaluation for the adequacy avoiding the cytopathologist, minimizing the costs and improving the educational benefit of physicians. (Civardi et al., 1988).

5.4.2 Type of needle and results of guided biopsy

Biopsies of the pancreas can be performed with needle ranging in size from 18 to 25 gauge (G). Aspiration biopsies for cytological evaluation are performed with fine-needle ($< 1\text{mm}$ in external diameter : from 20 to 25 G), cutting needle are used to obtain tissue cores, which allow histopathological evaluation, these needle ranging in size from 18 to 23 G.

In table 2 are reported the results of sensitivity, specificity, overall accuracy, method of guidance, needle size of percutaneous pancreatic fine-needle aspiration biopsy.

Our group (Di Stasi et al., 1998) in a multicenter study reviewed 510 patients who had a final diagnosis available and who had undergone ultrasound-guided fine needle biopsy of the

N° of patients	sensitivity (%)	specificity (%)	accuracy (%)	guidance	needle size	authors
510	87	100	95	US	21-22	Di Stasi et al (1998)
267	81	-	-	US	22	Bhatia et al (2008)
222	89	98	91	US	22 (20,25)	Garre Sanchez et al (2007)
104	77.9	100	81.7	US	-	Volmar et al; Zamboni (2005)
70	80	100	81	CT/US	-	Mallery et al (2002)
59	93	100	93	US	22	Matsubara et al (2008)
50	78.6	100	82	CT	-	Volmar et al (2005)
36	62	100	72	CT/US	20-22	Horwhat et al (2006)
545	99.4	-	99.4	US	21-22	Zamboni et al (2010)

Table 2. Sensitivity, specificity, accuracy method of guidance and needle size (gauge) of percutaneous fine needle aspiration biopsy of pancreatic masses

pancreas. Retrieval rate, sensitivity, specificity, and overall diagnostic accuracy of the whole series, by three different bioptic procedures (cytology, histology and cytology plus histology) were evaluated. The reliability of ultrasound-guided fine needle biopsy to allow a correct diagnosis in the different pancreatic pathologies was calculated for cytology, histology, and cytology plus histology, retrieval rate values were: 94%, 96%, and 97%; sensitivity was: 87%, 94%, and 94%, specificity:100%; and diagnostic accuracy: 91%, 90% and 95%, respectively.

In a series of 545 US-guided FNAB, 93,4% procedures were diagnostic, with an overall 99,4% sensitivity and 99.4% accuracy (Zamboni et al., 2010).

The largest series reporting in the literature percutaneous FNAB of pancreatic masses show sensitivities ranging between 62% and 93%, accuracies between 72% and 94%. The majority of percutaneous FNAB are ultrasound guided, and the needle size range from 21 to 22 G (Tab 2).

FNAB cytology of pancreatic cancer are reported in figure 1 a , b, 2 a, b, 3 a, b;

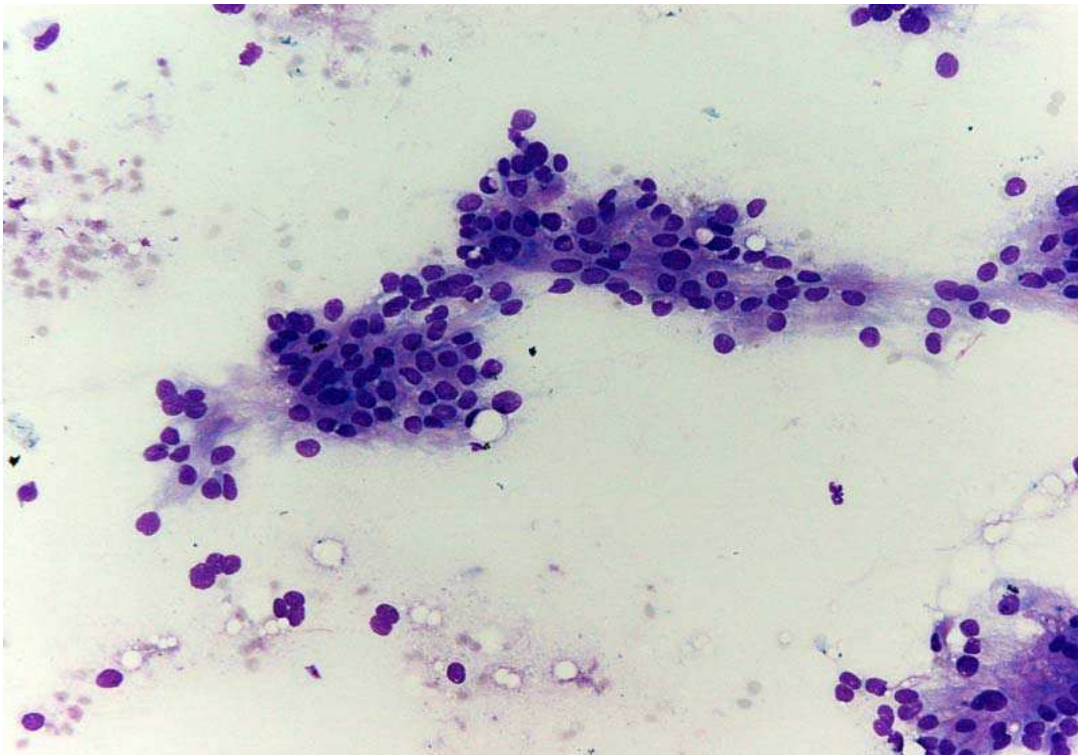


Fig. 1. a. FNAB of pancreatic well-differentiated adenocarcinoma, (a) relatively mild nuclear atypia, but nuclear crowding. MGG x 200

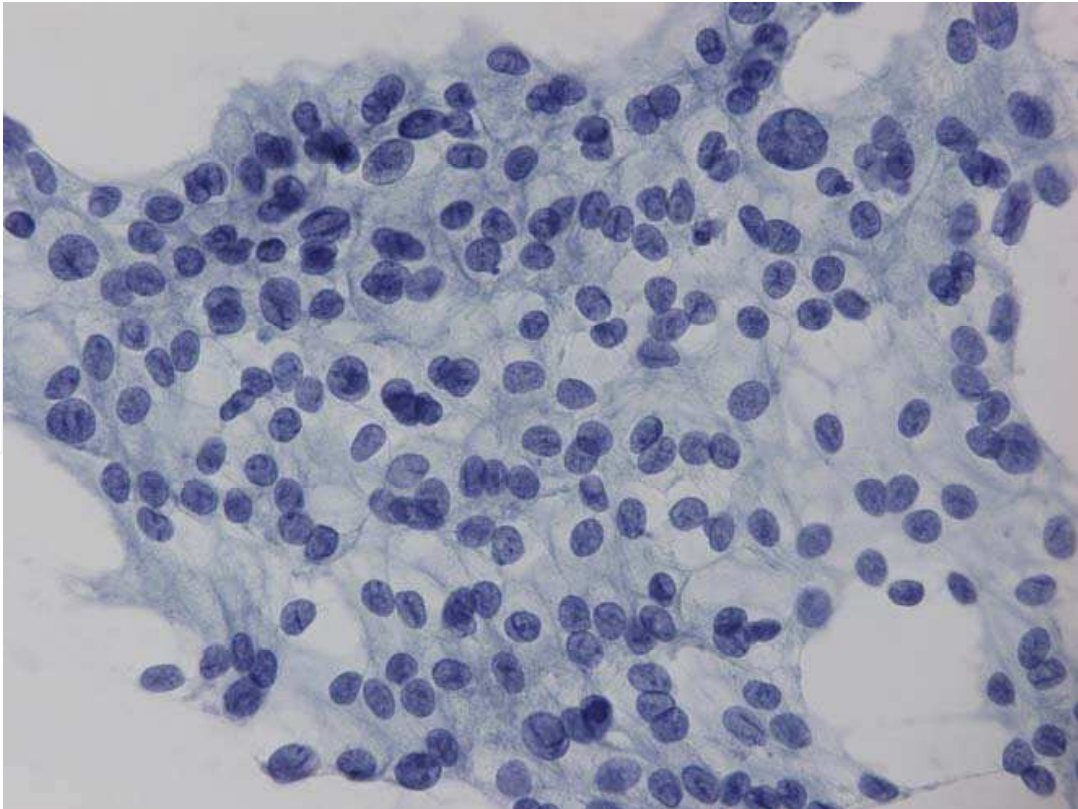


Fig. 1. b. Well evident microglandular arrangement. PAP x 400

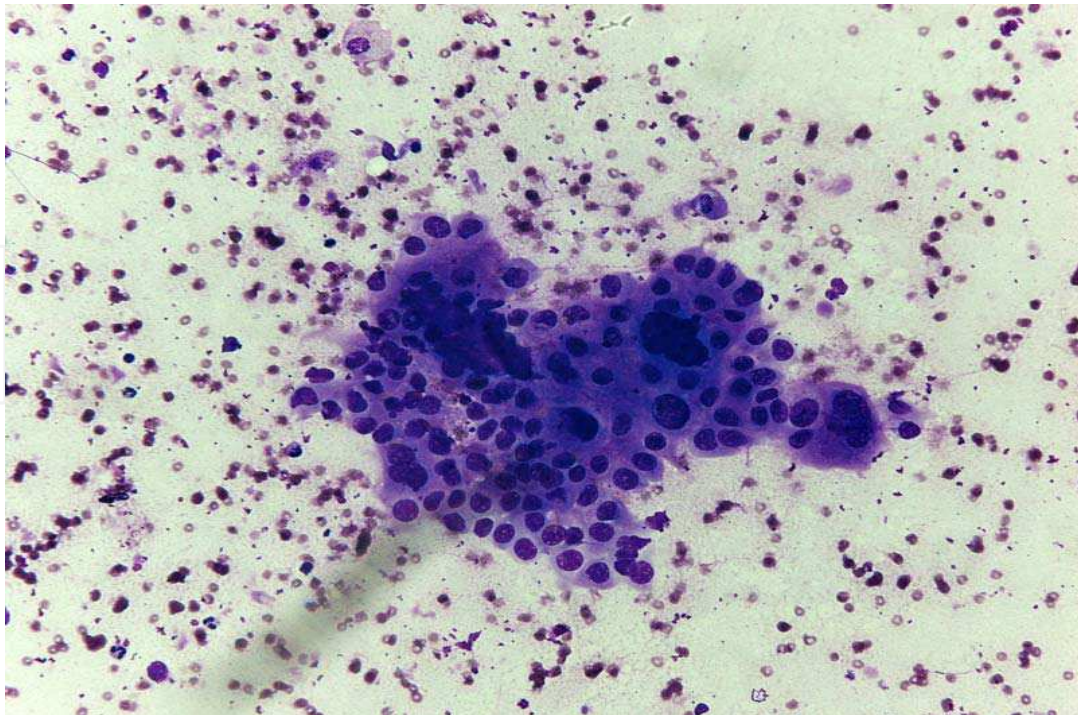


Fig. 2. a. FNAB of pancreatic moderately differentiated pancreatic adenocarcinoma. MMG x 200

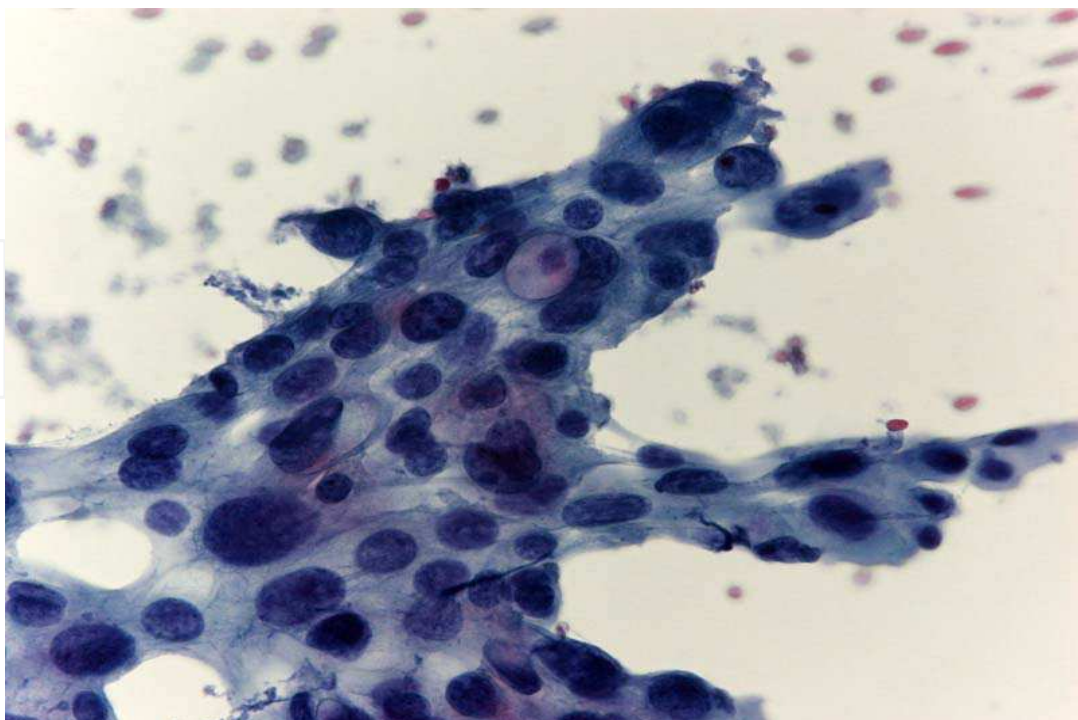


Fig. 2. b. Clusters of cells with some acinar arrangement. PAP x 400

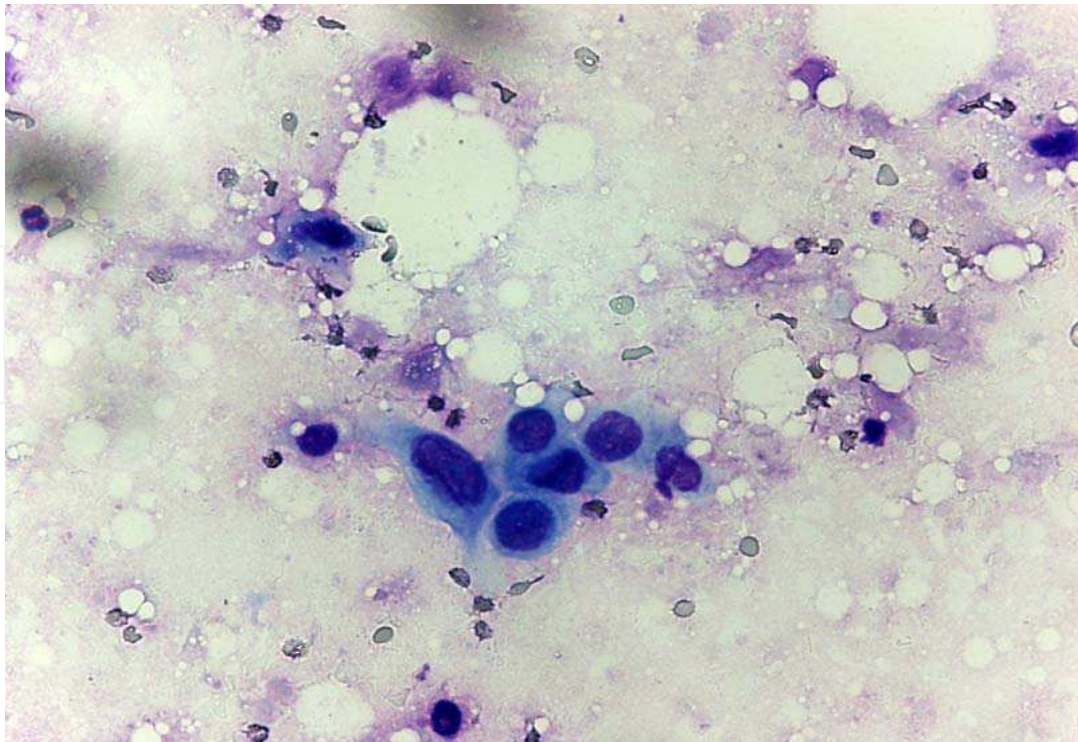


Fig. 3. a. FNAB of pancreatic poorly differentiated pancreatic adenocarcinoma, a cluster of cancer cells. MGG x 400

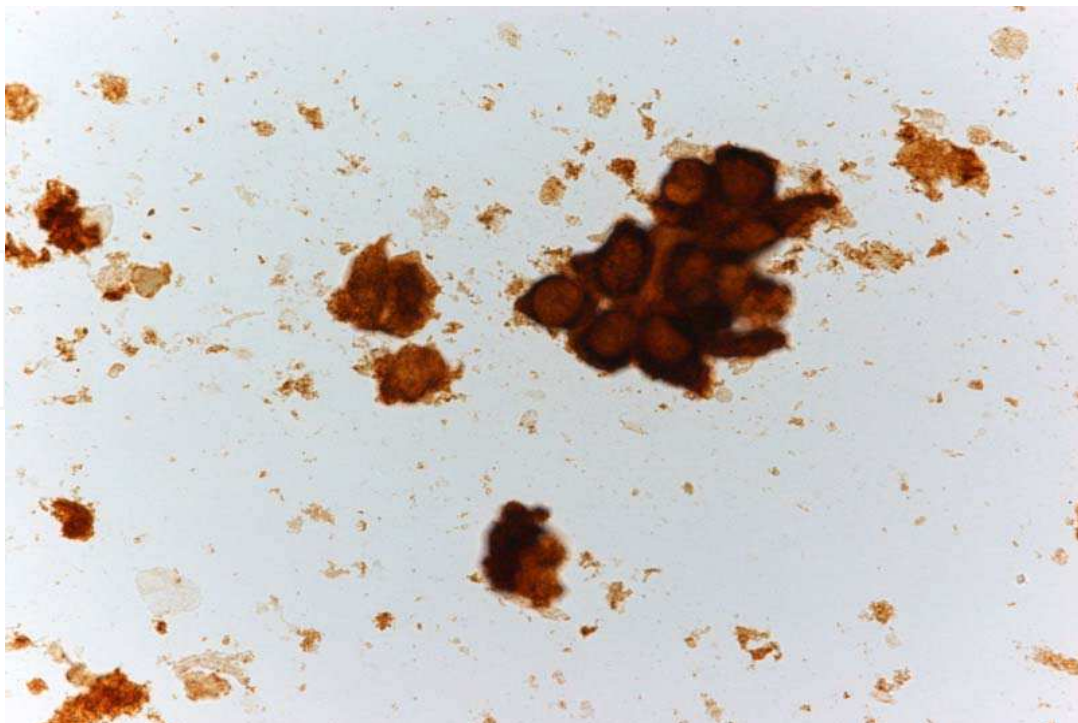


Fig. 3. b. Cancer cells with strong reactivity to CK 7 antibody. X 400

and FNAB cytology of pancreatic metastasis is reported in figure 4 a, b.

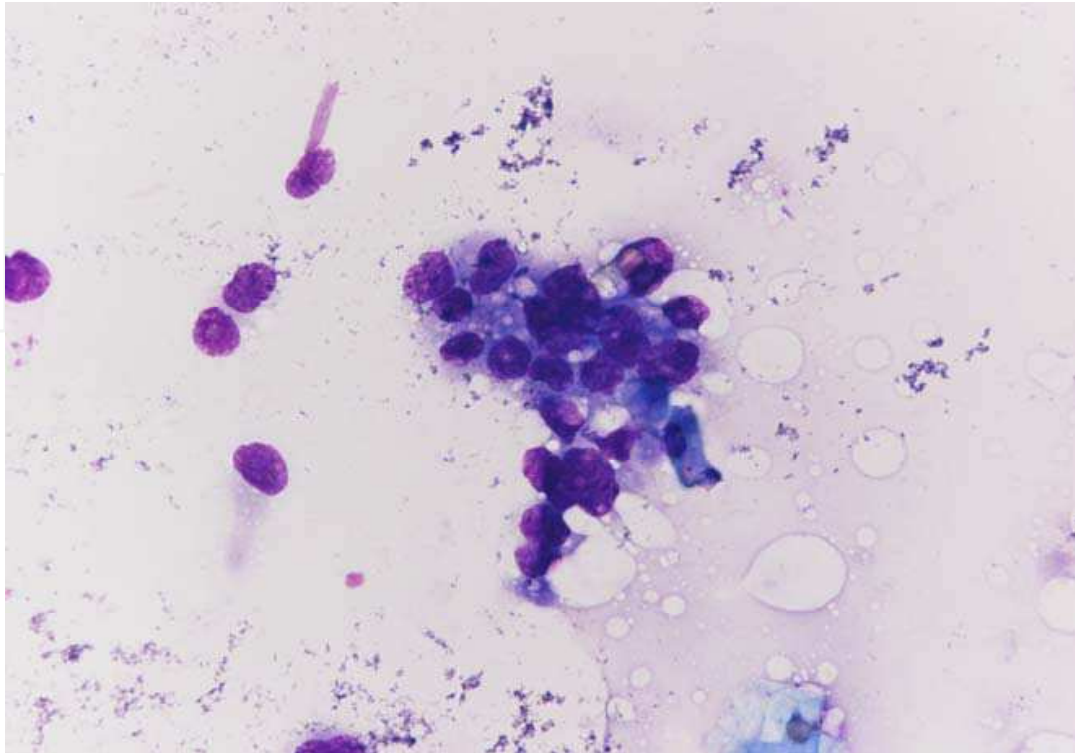


Fig. 4. a. FNAB of pancreatic mass showing metastatic melanoma large cells. MGG x 400

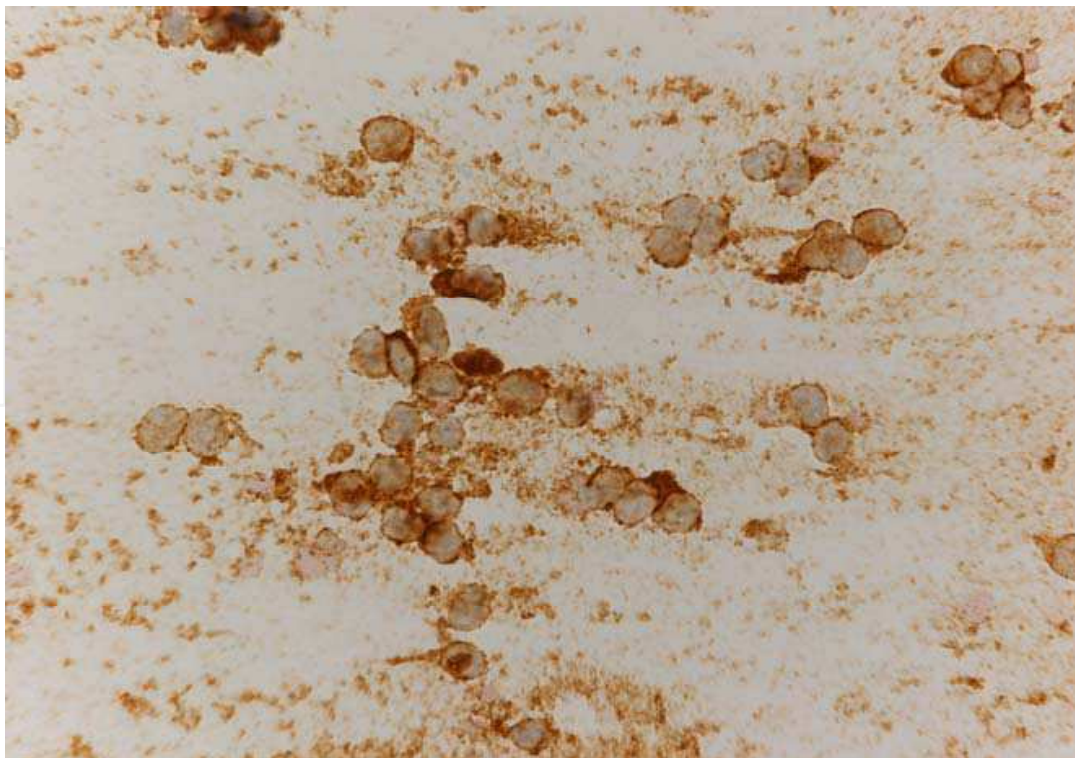


Fig. 4. b. Immunocytochemical HMB-45 positivity consistent with metastatic melanoma X 400

Sensitivity, specificity, accuracy method of guidance and needle size of percutaneous tissue core biopsy of pancreatic masses are reported in table 3.

N° of patients	sensitivity (%)	specificity (%)	accuracy (%)	guidance	needle size	authors
372	90	-	90	CT	18	Amin et al (2006)
212	86	100	86	US	21	Matsubara et al (2008)
142	90.9	-	92.6	US	-	Jennings et al (1989)
100	90	-	-	US	-	Karlson et al (1996)
92	92.5	100	93.3	US	18	Paulsen et al (2006)
50	90.4	-	92	US	-	Elvin et all (1990)
18	100	1	100	CT	18	Paulsen et al (2006)

Table 3. Sensitivity, specificity, accuracy method of guidance and needle size (gauge) of percutaneous tissue core biopsy of pancreatic masses

372 CT-guided pancreatic biopsies with a 18 G cutting needle showed 90% sensitivity and accuracy (Karlson et al., 1996); similar results were reported in a series of 212 US guided percutaneous tissue core pancreatic biopsies, with 86% sensitivity and accuracy (Paulsen et al., 2006).

The largest series in the literature on percutaneous tissue core biopsy of pancreatic lesions report sensitivities and accuracies between 86% and 100% (Tab 3). Tissue core biopsy of primary pancreatic lymphoma and metastatic adenocarcinoma are reported in fig 5 a b and 6 a b c. Percutaneous core biopsy of pancreatic lesions is considered sensitive, safe and accurate (Zanaboni et al., 2010) however this procedure may have a higher complication rate than percutaneous FNAB (Fornari et al., 1989).

At our institution we routinely use FNAB with 22G needles when a pathologic diagnosis of pancreatic mass is required while tissue core biopsy with 20G or 21G needles is reserved when cytological diagnosis is inadequate or when lymphoma is suspected on cytological evaluation (Arcari et al., 2005) Fig 5 a, b.

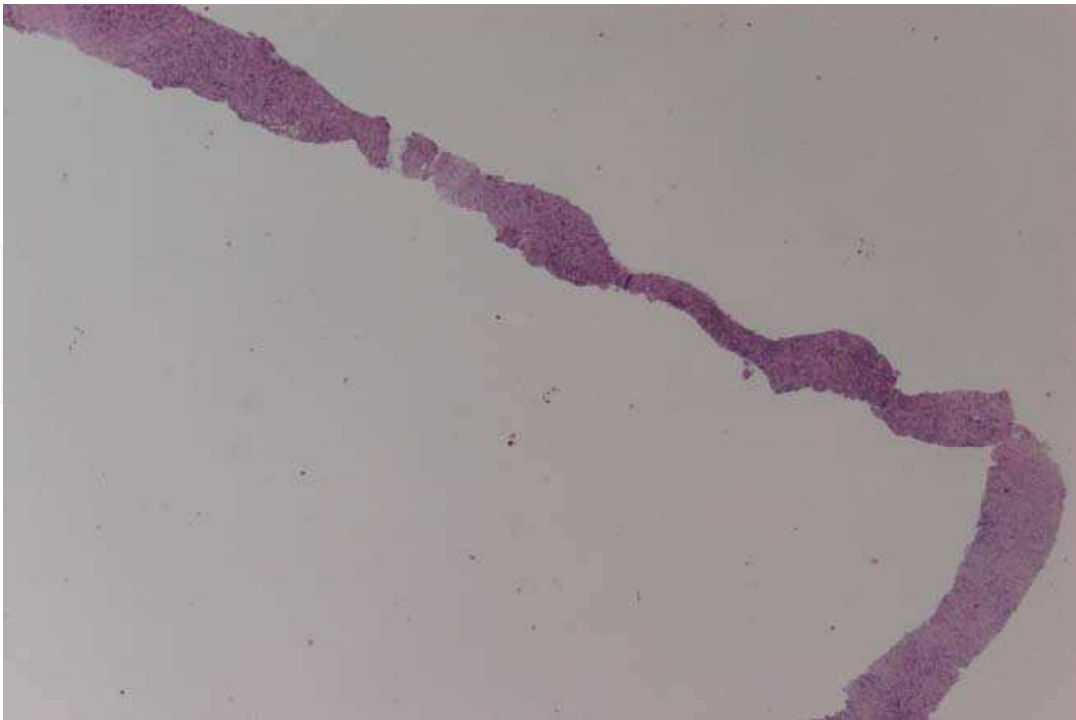


Fig. 5. a. Tissue core biopsy of a pancreatic mass. H&E X 20

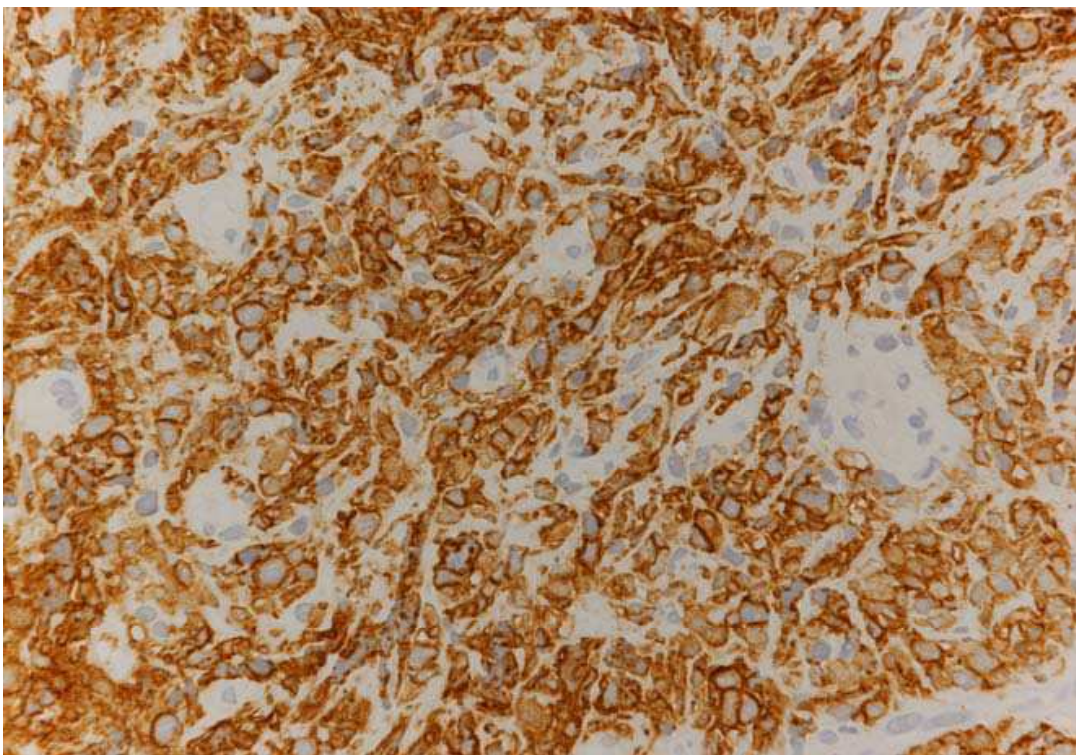


Fig. 5. b. Diffuse large B-cell Lymphoma CD20 positive. X 400

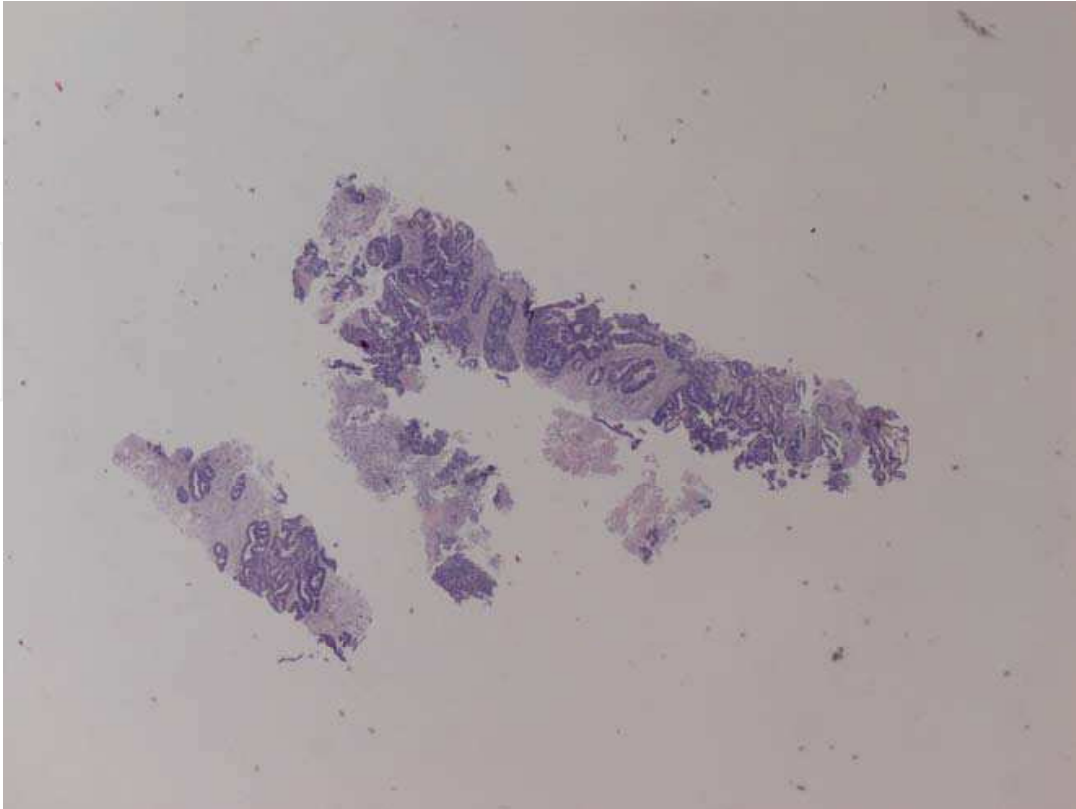


Fig. 6. a. Tissue core biopsy of a pancreatic mass. H&E x 20

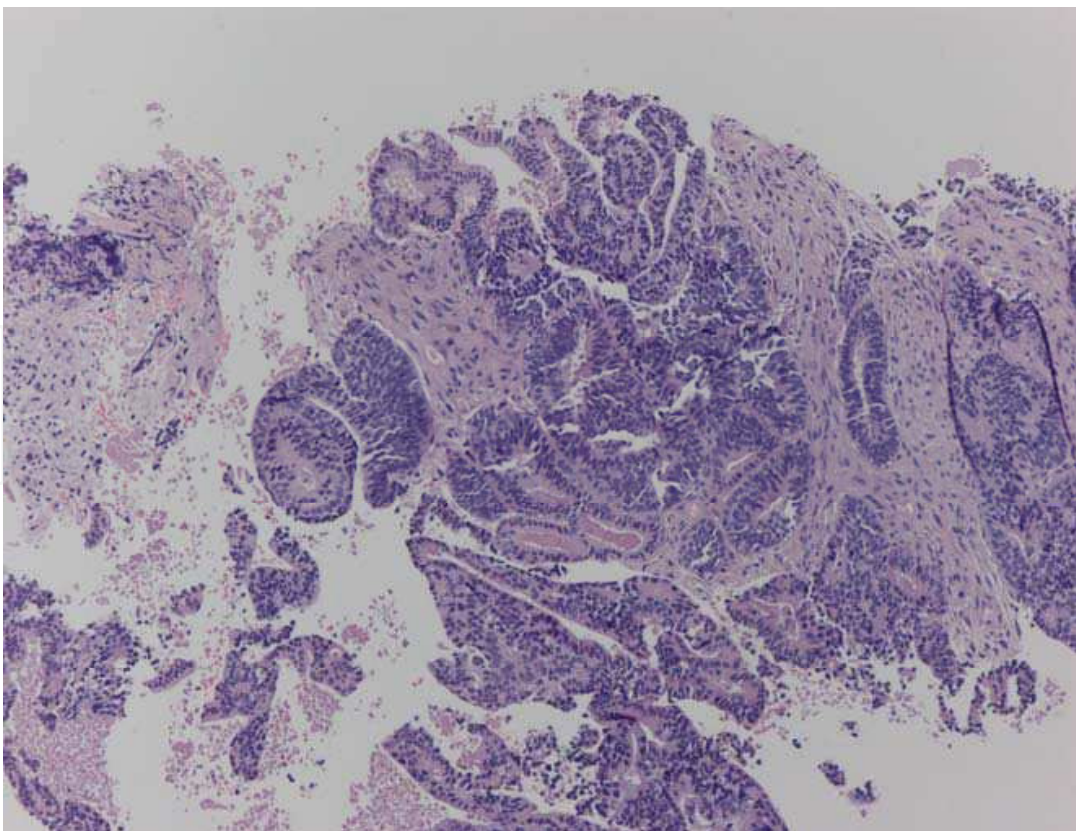


Fig. 6. b. Histology shows metastasis from endometrial adenocarcinoma. H&E X 100

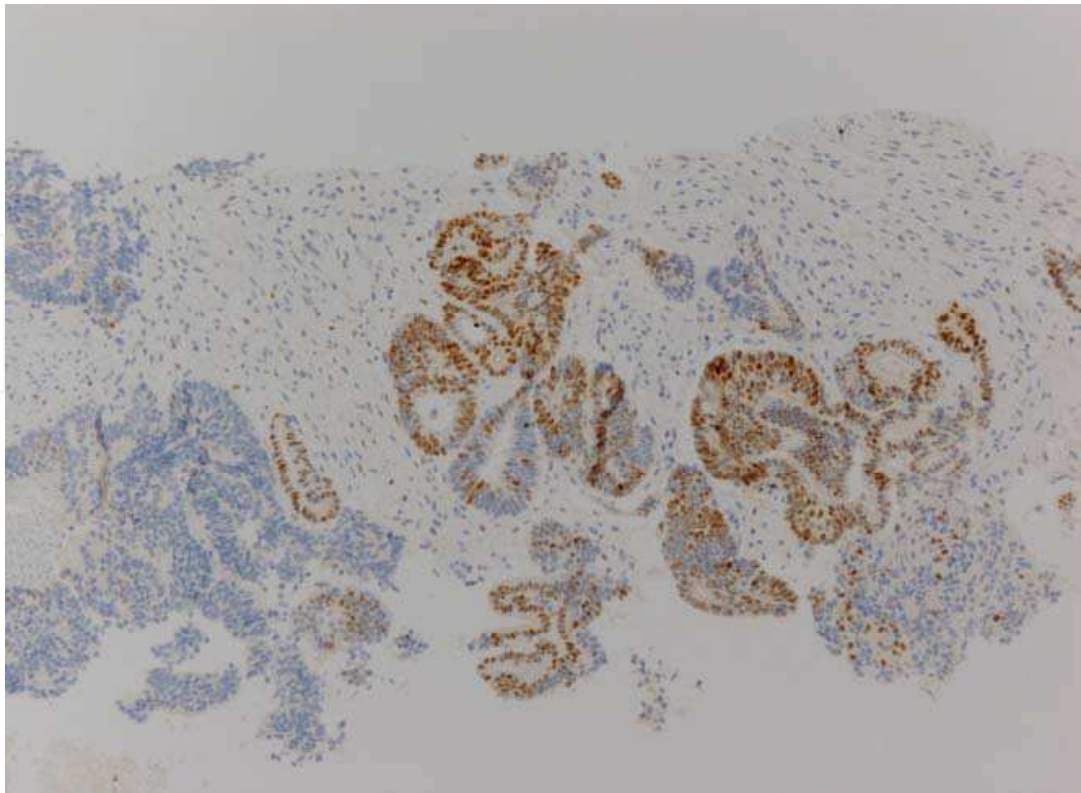


Fig. 6. c. Estrogen receptor positive neoplastic cells. X100

Since it was developed endoscopic ultrasound-guided fine needle aspiration biopsy has been widely used and has been adapted for gastrointestinal and perigastrointestinal lesions.

A medical literature review to evaluate the role of EUS-FNAB for diagnosis of pancreatic masses showed a 78-95% sensitivity, 75-100% specificity, 98-100% positive predictive value and a 78-95% accuracy (Yoshinaga et al, 2011) (Tab 4).

N of patients	Sensitivity %	Specificity %	Accuracy%	Needle size	Authors
583	84	86	84	22-26	Siddiqui et al (2011)
100	78	75	78	22	Touchefeu et al (2009)
182*	78.2-96.2*	98.4	86.2-96.8*	22	Garcia et al (2011)
737	77	99	80	22-25	Turner et al (2010)
207	92.6	88.6	91.8	22	Klimet et al(2010)

* The presence of an on site-cytopathologist was associated with a significantly higher diagnostic sensitivity and overall accuracy for malignancy.

Table 4. Sensitivity, specificity, accuracy needle size of endoscopic-guide pancreatic biopsy

6. Complications of pancreatic biopsy

US, EUS or CT guided fine-needle biopsy are considered to be a low risk procedure.

Interventions with needle with a larger diameter seem cause more complications.

The major complication of pancreatic biopsy can be hemorrhage, needle track seeding and pancreatitis.

Our group (29) reported the complications following 10.766 US-guided fine-needle abdominal biopsies. The mortality was 0.018%: the two reported deaths were due to hemoperitoneum and occurred in patients with hepatocellular carcinoma arising in cirrhotic liver.

The biopsy of pancreatic carcinoma was more dangerous for needle –track seeding (five of eight reported cases), however, it has been reported that peritoneal carcinomatosis may occur more frequently in patients who undergo percutaneous FNAB compared with those who have FUS-FNAB for the diagnosis of pancreatic cancer (Micames et al, 2003).

6.1 Conclusions

There is consensus in the literature of the appropriateness of obtaining a cytohistological diagnosis in patients with unresectable pancreatic neoplastic lesion, prior to initiate chemotherapy and/or radiation.

Although the American Joint Committee on Cancer has selected EUS-guided FNAB as the procedure of choice, if available, we recall that there is wide variability in the world on the modalities for guide biopsy (US ; CT ; EUS) and for needle biopsy choice (FNAB or tissue core biopsy).

There is a consensus that local expertise, the availability of EUS and interventional percutaneous procedures may determine the choice for pancreatic biopsy.

In agreement with other authors (Zamboni et al., 2010) at our institution , in the appropriate setting, percutaneous US-guided FNAB is considered the first invasive approach of obtaining tissue diagnosis confirmation in patients with unresectable lesions.

However, guided FNAB or guided tissue core biopsies remain invasive procedures and must be performed when informations so obtained benefits the patient.

7. Acknowledgments

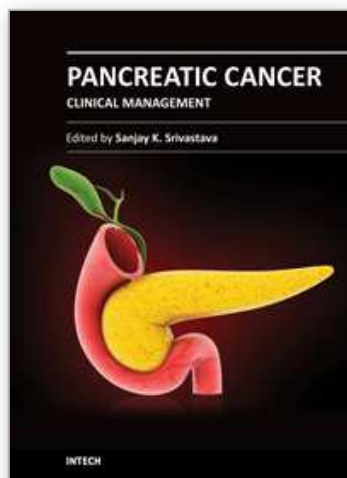
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This book covers pancreatic cancer risk factors, treatment and clinical procedures. It provides an outline of pancreatic cancer genetic risk factors, biomarkers and systems biology for the better understanding of disease. As pancreatic cancer suffers from lack of early diagnosis or prognosis markers, this book encompasses stem cell and genetic makers to identify the disease in early stages. The book uncovers the rationale and effectiveness of monotherapy and combination therapy in combating the devastating disease. As immunotherapy is emerging as an attractive approach to cease pancreatic cancer progression, the present book covers various aspects of immunotherapy including innate, adaptive, active, passive and bacterial approaches. Management of anesthesia during surgery and pain after surgery has been discussed. Book also takes the reader through the role of endoscopy and fine needle guided biopsies in diagnosing and observing the disease progression.

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