Percutaneous fine needle aspiration cytology of the pancreas: advantages and pitfalls

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SUMMARY Fine needle aspiration of the pancreas was performed in 62 patients with radiological suspicion of malignancy. All fine needle aspirates were taken under computed tomography or ultrasound guidance. Fine needle aspirates were positive in 31 of 41 patients with histologically or clinically confirmed pancreatic carcinoma. There were no false positive results. The sensitivity of this method for detecting malignant disease was 86%. Cytology was not able to provide conclusive results of benign conditions. Difficulties were encountered in diagnosing well differentiated carcinoma and neuroendocrine tumours and distinguishing them from reactive epithelium and islet cell hyperplasia, respectively. This resulted in a 12.1% false negative rate. There were no complications in our series. Percutaneous fine needle aspiration proved a reliable method of diagnosing pancreatic carcinoma.

The primary objective of the doctor caring for a patient whose symptoms suggest pancreatic carcinoma should be to establish the diagnosis with a minimum of time, testing, money and suffering.¹ The mortality of patients with this disease is 99% within the first two years.² Early diagnosis remains the only known method of cure. Fine needle aspiration cytology of the pancreas under radiological guidance,³⁻¹³ or during surgery,¹⁴⁻¹⁶ has been widely used as a diagnostic method. It is especially useful in diagnosing solid neoplasms which account for over 90% of pancreatic tumours.¹⁶

Material and methods

Fine needle aspirates of the pancreas were taken using a 21G spinal needle under computed tomography guidance and local (subcutaneous) anaesthesia. The needle was attached to a 20 ml syringe and suction enhanced by using a syringe holder. Aspiration was performed after reaching the desired site by applying repeated suction and by releasing it before the needle was withdrawn from the lesion. This prevented the negative pressure forcing the blood into the syringe. After removing the needle from the patient the needle was detached from the holder and aspirated material was ejected on to glass slides averaging nine smears in each case. Cystic fluids and needle washouts were transported in buffered saline.

The fluids were processed in the cytocentrifuge. All

slides were either air dried or fixed in alcohol and stained by either May-Grünwald-Giemsa or Papanicolaou methods, respectively. Whenever possible, spare slides were kept frozen at -20°C for immunocytochemical analysis. Carcinoembryonic antigen (CEA), neuron specific enolase (NSE), and chromogranin were the antibodies used in a limited number of cases.

Results

Fine needle aspiration cytology was performed in 62 patients with radiological or clinical evidence of pancreatic mass. Definitive diagnosis of carcinoma from fine needle aspirate smears was made in 31 patients. In 21 patients this was confirmed by the histological core biopsy specimen taken at the same time, and in 10 patients clinically by the rapid progress of the disease. There were no false positive results. Cytology was negative in 17 patients; in seven patients material was inadequate for cytological assessment due to poor cell yield or preservation. In seven patients aspirates were suspicious but not diagnostic. Subsequent histology showed carcinoma in five of the negative and two of the inadequate aspirates. Patients with findings suspicious but not diagnostic of malignancy diagnosed in cytological smears were biopsied. Biopsy specimens showed carcinoma in three cases, were negative in two, inadequate in one and suspicious of malignancy in one case. The overall sensitivity of fine needle aspiration cytology in diagnosing pancreatic carcinoma was 86%, excluding the cases where

Table Correlation between cytological and histological diagnosis, excluding suspicious and inadequate specimens, with final clinical outcome (Figures in parentheses are cases with clinical or clinical and cytological diagnosis only)

Definitive diagnosis	Cytology			Histology		
	No of cases	Sensitivity (%)	Specificity (%)	No of cases	Sensitivity (%)	Specificity (%)
Benign	21 (5)	17		16		
Malignant	41 (10)	31	86	31	89	100
Total	62	48*		47†		

*Results exclude seven inadequate and seven suspicious aspirates.

†Results exclude 11 inadequate and four suspicious biopsies.

diagnosis was suspicious and not diagnostic of malignancy (table).

CYTOLOGICAL FEATURES

In addition to normal pancreatic cells, fine needle aspirates often contained some of the cells from adjacent anatomical structures. Their features are listed below:

Acinar cells (fig 1): These often appear in clusters with round nuclei and finely granular chromatin pattern. Nucleoli are usually small but can be conspicuous. Cytoplasm is abundant, well outlined, but can often be missing, leaving bare nuclei.

Ductal cells (fig 2): These are of cuboidal type, often in tightly cohesive flat sheets resembling ducts. Cytoplasm can contain secretory vacuoles.

Mesothelial cells: These occur in flat sheets with prominent intercellular spaces. Nuclei are uniform, centrally placed, and often contain nucleolus.

Endothelial cells: Fragments of blood vessels are easily recognisable as elongated cells in parallel lines, often containing red blood cells.

Hepatocytes: These are cuboidal cells, often appearing in flat sheets, have central nucleus, prominent nucleolus and abundant granular cytoplasm containing pigment.

Intestinal epithelium: This often appears as sheets of columnar epithelium showing clear luminal border.

Cytological criteria for the diagnosis of pancreatic carcinoma,¹⁷ listed in order of relevance, are:

1 Extreme nuclear enlargement with nuclear contour irregularity.

Fig 1 Pancreatic fine needle aspirate smear: acinar cells. (Papanicolaou stain, oil immersion.)

Percutaneous fine needle aspiration cytology of the pancreas

Fig 2 Pancreatic fine needle aspirate smear: duct cells. (May-Grünwald-Giemsa stain.)

Fig 3 Pancreatic fine needle aspirate smear: well differentiated adenocarcinoma, erroneously interpreted as duct cell atypia. (May-Grünwald-Giemsa.)

Fig 4 *Pancreatic fine needle aspirate smear: poorly differentiated carcinoma showing classic features of malignancy. (May-Grünwald-Giemsa.)*

Fig 5 *Pancreatic fine needle aspirate: islet cells in islet cell hyperplasia. (Papanicolaou stain.)*

Fig 6 Pancreatic fine needle aspirate smear: islet cell carcinoma (May-Grünwald-Giemsa.)

- 2 Disoriented or crowded cells in three dimensional groups.
- 3 Unevenly distributed chromatin.
- 4 Anisonucleosis.
- 5 Raised nucleus: cytoplasm ratio.
- 6 Prominent nucleoli.

Cell typing of malignancies seen in fine needle aspiration smears showed all to be adenocarcinomas. It was usually possible to categorise these according to the degree of differentiation into well, moderately, and poorly differentiated adenocarcinomas (figs 3 and 4). One tumour was classified as mucinous cystadenocarcinoma. One of the cytologically negative smears was a metastasising islet cell tumour, interpreted as islet cell hyperplasia (figs 5 and 6).

Immunocytochemistry (APAAP) was performed in a few cases where not all smears were used for routine staining. Difficulties were encountered with strong background staining on the smears. Similarly, heavy admixture of blood often prevented recognition of the relevant cells in the immunostained preparations. As material was usually received both fixed in alcohol and unfixed we were left with the limited choice of the optimal (unfixed) material. The best results were obtained from cell solutions where cells were washed and cytospin preparations resulted in considerably less background staining.

Most of the carcinomas showed strong cytoplasmic CEA positivity. Normal pancreatic ducts and intes-

tinal epithelium, however, showed similar but weaker staining. We did not use immunocytochemistry as a distinguishing criterion between benign and malignant lesions. Some of the smears from tumours that showed histological evidence of neuroendocrine differentiation were destained and stained for NSE and chromogranin. The results were not satisfactory due to poor preservation of antigen in destained smears.

Discussion

The main role of fine needle aspiration cytology of the pancreas is in differentiating benign from malignant conditions. The method has been proved reliable^{3 10 18-21} and safe.²²⁻²⁶ In a series of more than 3000 fine needle aspirates Kline *et al* experienced no complications.²² Similar experience was also described by Ho in a series of 1500 fine needle aspirates²³ and in reports by Holm *et al*,²⁴ Zajicek,²⁵ and Engzell *et al*²⁶ on several hundred patients with malignant tumours.

Recent advances in imaging techniques have improved the methods of localisation of these tumours.²⁷ The sensitivity of the method in diagnosing malignant disease of the pancreas varied in the reported series from 50–86%.²⁸⁻³⁸ In the present series we found sensitivity of fine needle aspiration to be 86%, disregarding suspicious results.

In our experience the main limitations of fine needle aspiration cytology of the pancreas are:

False negative results These are due to poor sampling, technical preparation, and interpretation. Poor sampling can be due to scarring and fibrosis associated with some tumours. It can also be due to the needle missing a small neoplasm. Two of the inadequate specimens in our series were subsequently proved malignant. Technical preparation of direct smears was difficult because of the heavy admixture of blood in most specimens, causing early clotting and preventing proper spreading of the smear. The cells were trapped in the clot and were difficult to interpret. Problems of interpretation can occur when distinguishing epithelial atypia due to chronic pancreatitis or duct obstruction from well differentiated neoplasms, particularly when smears are poorly cellular and contain a high admixture of blood. Five of the cases reported as benign or reactive proved malignant (fig 3). Similar difficulties were met in three of the seven cytologically suspicious lesions which subsequently proved malignant. The false negative rate in our series was 12.1%.

Diagnosis of neuroendocrine cell tumours One of the cytologically benign cases in our series was a malignant neuroendocrine tumour with subsequent metastases in the liver. Cytological smears showed remarkably uniform islet cells in aggregates (fig 6). As these are sometimes seen in islet cell hyperplasia (fig 5) accompanying chronic pancreatitis, they were interpreted as benign. The experience of some authors shows that cell morphology alone is sometimes sufficient for diagnosing these tumours.^{39 40}

Differentiation of cell type Most of the tumours in this series were adenocarcinomas, showing various degrees of differentiation. Cytologically we were able to classify one of the tumours as mucinous cystadenocarcinoma. It was not possible, however, to detect foci of neuroendocrine differentiation within individual tumours and report with confidence tumours showing histologically two components (adenosquamous carcinoma). Whether the separation of these histological types will eventually prove relevant to some epidemiological clinical types, clinical feature, or to a response to a specific chemotherapeutic regimen is not known.¹

The most important question to be answered by fine needle aspiration cytology is whether a carcinoma is present or not. Using the criteria described by Mitchell *et al.*,¹⁷ we feel that extreme nuclear enlargement and disoriented cells in three dimensional clusters are the most helpful features for distinguishing between benign and malignant lesions, regardless of the quantity of cells and presence of other features described by the same authors. In this respect aspiration cytology is a reliable method on which to base a decision to proceed with radical operation in selected cases, as well as prognosis in inoperable cases.

Fine needle aspiration cytology is a quick, safe, and

reliable method of diagnosing pancreatic carcinoma. It is inexpensive and is an easily tolerated procedure. Only positive results are of value and negative results do not exclude malignancy.

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