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Endoscopic ultrasound-guided fine needle aspiration of the pancreas: cytomorphological evaluation with emphasis on adequacy assessment, diagnostic criteria and contamination from the gastrointestinal tract¹

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Endoscopic ultrasound-guided fine needle aspiration of the pancreas: cytomorphological evaluation with emphasis on adequacy assessment, diagnostic criteria and contamination from the gastrointestinal tract

Objective: Endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) has been proved to be safe, efficient and reliable in the diagnosis of pancreatic lesions. This study evaluated specimen adequacy, diagnostic criteria of various pancreatic neoplasms and contamination from the gastrointestinal (GI) tract.

Methods: EUS-guided FNA of the pancreas and subsequent surgical resections performed at the University of California Irvine Medical Center during February 1996–October 2000 were retrospectively selected. Modified Papanicolaou staining method was used for immediate evaluation and cell block prepared.

Results: A total of 267 cases were available for review, including 147 (55.1%) positive/suspicious, 10 (3.7%) atypical, 96 (36.0%) negative and 14 (5.2%) unsatisfactory cases. Eighty-six (58.5%) positive/suspicious cases had histological confirmation and 12 (8.3%) had lymph node or distant metastases by cytology. Three atypical, two negative, and two unsatisfactory cases proved to have adenocarcinoma. Contamination from duodenum, stomach or pancreas was found in 77 positive/suspicious, three atypical and 90 negative cases. The sensitivity, specificity, diagnostic accuracy, positive and negative predictive values were 94.6%, 100%, 95.6%, 100%, 82% respectively.

Conclusions: EUS FNA is efficient and accurate in the diagnosis of pancreatic neoplasms in adequate samples. Contamination from the GI tract should be well recognized to avoid misinterpretation.

Keywords: endoscopic ultrasound, fine needle aspiration, cytology, pancreas, EUS FNA, EUS FNAC

Introduction

Endoscopic ultrasound (EUS) was first introduced 22 years ago and has become established as an

accurate means for staging GI and pancreatic malignancies with an accuracy of approximately 90%.^{1–3} However, being a pure imaging modality, it was difficult to distinguish metastatic lymph nodes from reactive lymph nodes and pancreatic cancer from pancreatitis. In other words, the test is very sensitive, but lacks specificity (reported to be 50–70%).⁴ The application of fine needle aspiration (FNA) to a linear array echoendoscope has dramatically expanded the clinical utility of EUS in that cytological material can be obtained while the needle is visualized on real-time ultrasonography.^{4–12} However, only a few studies have been found in the cytology literature that evaluated the cytological features in specimens obtained from pancreatic tumours by EUS-guided FNA.^{13–16} In

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addition, adequacy assessment and contamination from the gastrointestinal (GI) tract and pancreas, unique to this diagnostic modality, have not been sufficiently emphasized. The current study was carried out retrospectively to evaluate specimen adequacy, the cytological features of various pancreatic tumours and to emphasize the importance of recognizing the contamination from the GI tract and pancreas that may compromise the diagnostic interpretation in specimens obtained by EUS-guided FNA.

Methods

During February 1996–October 2000, EUS-guided FNA biopsies of the pancreas performed at the University of California Irvine Medical Center were retrospectively retrieved by a computer search from the files of the Cytology Service in the Department of Pathology. All biopsy procedures were performed in the Endoscopy Suite. FNA was performed by using a 22-gauge, 10-cm needle (Wilson-Cook, Wilson-Cook needle; Winston-Salem, NC, USA) or a GIP-Medi-globe needle (Temple, AZ, USA). The aspirated samples were expelled onto slides, and two smears were made for each aspiration pass, followed by fixation in 95% alcohol. Multiple passes, if necessary, were done in order to obtain diagnostic material.

The slides were then immediately transported to the Cytology Service for adequacy evaluation and preliminary interpretation. Modified rapid Papanicolaou staining method (Richard Allen Scientific, Kalamazoo, MI, USA) was used for this purpose. The number of passes until satisfactory specimens were obtained was documented in each case. Cell block material was obtained by a separate pass in each patient and was fixed in 10% buffered neutral formalin. Thin sections (4 µm) from paraffin-embedded cell block were cut on the following day and were stained with haematoxylin and eosin (H&E). On retrospective review of the cytological slides, the following features were systematically analysed in each case: cellularity, presence of sheets or loosely cohesive aggregates or discohesive single tumour cells, quality and quantity of cytoplasm, nuclear pleomorphism, chromatin patterns, nucleus to cytoplasm (N/C) ratio, necrosis and presence of tumour cells in cell blocks. Cellularity was determined arbitrarily by the average number of tumour cells at any 10× magnification. If more than 50 tumour cells either singly or in clusters could be identified, the case was regarded as high cellularity. If less than 50 tumour cells could be seen, then the case

was categorized as low cellularity. Chromatin patterns were either fine or coarse and either evenly or unevenly distributed.

For patients who had subsequent surgical resections or autopsies, tissues were fixed in 10% buffered neutral formalin. Thin histological sections (4 µm) were cut from paraffin-embedded tissue blocks followed by H&E stain. All histological slides were retrospectively reviewed.

Results

In total, 298 EUS-guided FNA procedures were performed at UCIMC in 291 patients during the study period. Two hundred and sixty-seven cases in 264 patients were available for retrospective review. They included 147 (55.1%) cases that were positive/suspicious for malignancy, 10 (3.7%) cases that were atypical, 96 (36%) cases that were negative for malignant cells and 14 (5.2%) cases that were unsatisfactory for evaluation due to scanty, acellular or bloody specimens. The mean age of the patients was 67.6 years (range 26–87). One hundred and forty-six patients were males and 128 were females. The positive/suspicious category included 140 adenocarcinomas, three endocrine tumours, two malignant lymphomas, one solid pseudo-papillary tumour and one plasmacytoma. Among these patients, 86 (58.5%) had confirmatory histology (including all 14 cases that were suspicious for adenocarcinoma) and 12 (8.3%) had cytological evidence of either lymph node or distant metastases. Twenty-four patients had clinically unresectable adenocarcinoma and received adjuvant therapy after the procedure and 25 patients returned to their referring medical facility for treatment and no further follow-up information was available for this study. In 122 patients with sufficient follow-up information, no false-positive cases were found (Table 1). The average number of aspiration passes necessary for an immediate interpretation as being positive or suspicious for malignancy was 4 (range 1–12).

In 10 patients with atypical diagnoses, three proved to have adenocarcinoma on subsequent surgical specimens. Three patients had repeat EUS-guided FNA and were all negative. Chronic pancreatitis was diagnosed on biopsy in two patients and on resection in two patients. In 96 cases with negative diagnoses, 21 (19.8%) patients had undergone surgical procedure, adenocarcinoma was found in two patients and pancreatitis was found in 19 patients. Seventy-five patients returned to their referring medical facility for

Follow-up	Category			
	Positive/ suspicious	Atypical	Negative	Unsatisfactory
Biopsy/resection (%)	147 (55.1)	10 (3.7)	96 (36)	14 (5.2)
Adenocarcinoma	90	3	2	2
Endocrine tumour	3	0	0	0
Lymphoma	2	0	0	0
SPT	1	0	0	0
Plasmacytoma	1	0	0	0
Pancreatitis	0	7	19	6
Lost	25	0	75	6
Total	122	10	21	8

Table 1. Results of 267 EUS-guided FNA biopsies with 161 follow-up patients

EUS, endoscopic ultrasound; FNA, fine needle aspiration; SPT, solid pseudo-papillary tumour.

management and no further follow-up information was available. In 14 unsatisfactory cases, surgical biopsy was performed in eight patients, including six patients with chronic pancreatitis and two patients with adenocarcinoma. No follow-up information was available in the remaining six patients who had unsatisfactory results (Table 1). For statistical analysis, the positive/suspicious category was used as true positive group and the rest (atypical, negative, and unsatisfactory) regarded as the negative group. A total of 161 patients had sufficient follow-up information for statistical analysis. The sensitivity and specificity were 94.6% and 100% respectively. The positive and negative predictive values were 100% and 82% respectively. The diagnostic accuracy was 95.6%.

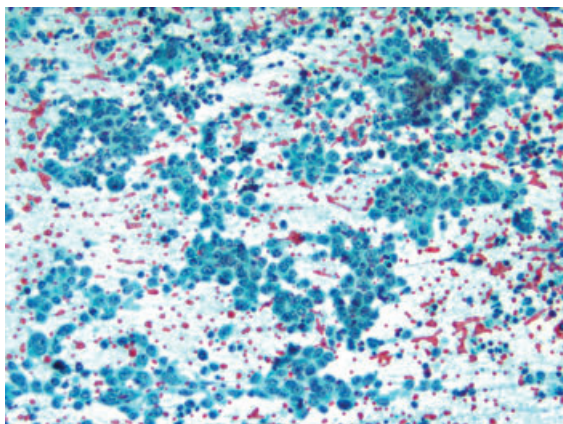


Figure 1. High cellularity. At 10× magnification, the tumour cells are present in all quadrants of the microscopic field (modified Papanicolaou stain, 10×).

In 147 positive/suspicious cases, 36 (24.5%) were classified as high cellularity (Figure 1) and 111 (75.5%) classified as low cellularity (Figure 2). Ten atypical cases were all low cellularity. In 96 negative cases, 27 were highly cellular and 69 of low cellularity. Cell block was available in 114 positive/suspicious cases and contained diagnostic material in 91 (61.9%) cases. In addition, cell block was exclusively diagnostic in 18 (20%) cases, in which cases the smears were not. Eight of 10 atypical and 78 of 96 negative cases had good cell blocks that supported the interpretation of the smears. In seven false-negative cases (three atypical, two negative, and two unsatisfactory), retrospective review of the slides confirmed the initial interpretation and were either

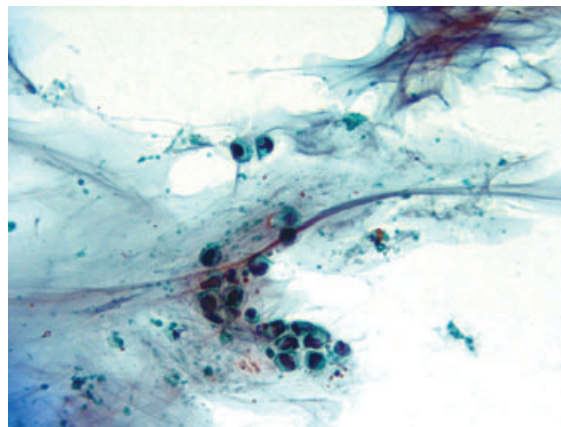


Figure 2. This is a low cellularity sample of a well-differentiated mucinous adenocarcinoma, characterized by abundant extracellular mucin and a few discohesive tumour cells with signet ring cell morphology (E, 20×) (modified Papanicolaou stain).

of low cellularity or acellular, with an average of four passes.

Cytological features

Adenocarcinoma. In 140 cases that were classified as adenocarcinoma, 111 cases were hypocellular and 29 cases were hypercellular. Disarrayed cohesive sheets of tumour cells were predominantly present in 20 (14.3%) cases. Abnormal cellular arrangement with 3-dimensional balls, prominent in 86 (61.4%) cases associated with detached single cells, were important features for well-differentiated adenocarcinoma (Figure 3a). Small loosely cohesive aggregates of tumour cells (Figure 3b) were predominant in 95 (67.9%) cases. Single cells, which essentially were seen in all malignant cases, were predominant in 25

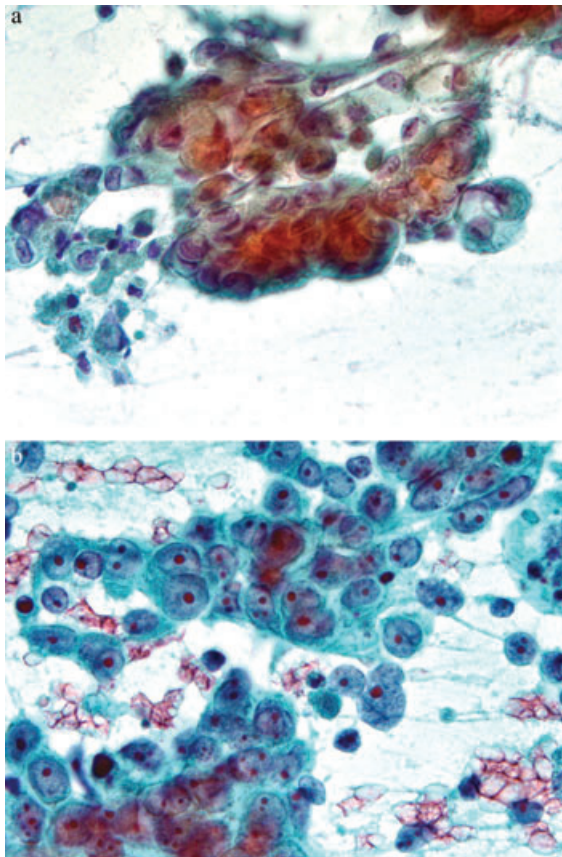


Figure 3. Adenocarcinoma. On high magnification, abnormal cellular arrangements with three-dimensional balls were noted with a few single cells at the edge (a). Loosely cohesive aggregates of tumour cells were predominant in this case and notice the prominent eosinophilic macronucleoli (b) (40 \times , modified Papanicolaou stain).

Table 2. Summary of cytological features of adenocarcinoma (140 cases)

Architecture	
Hypocellular	111 (79.3)
Hypercellular	29 (20.7)
Cohesive sheets	20 (14.3)
Small loose aggregates	95 (67.9)
Three-dimensional balls	86 (61.4)
Predominantly single cells	25 (17.8)
Cytomorphology	
Nuclear features	
Prominent nucleoli	98 (70)
Coarse hyperchromatic chromatin	118 (84.2)
Irregular membrane	127 (90.7)
High N/C ratio	86 (61.4)
Marked pleomorphism	81 (57.6)
Cytoplasm	
Moderate to abundant	54 (38.6)
Scanty	86 (61.4)
Vacuolated	129 (92.1)
Background	
Tumour diathesis (focal or diffuse)	126 (90)
Clean	14 (10)

Values within parentheses are expressed in percentage.

(17.8%) cases. Enlarged round nuclei with prominent eosinophilic nucleoli were conspicuous in 98 (70.0%) cases (Figure 3b). Coarse unevenly distributed hyperchromatic chromatin, irregular nuclear membranes, and tumour cells with high N/C ratio were seen in 118 (84.2%), 127 (90.7%) and 86 (61.4%) cases respectively. Marked nuclear pleomorphism was noted in 81 (57.6%) cases and necrosis (diffuse or focal) was seen in 126 (90.0%) cases.

The cytoplasm was moderate to abundant in 54 (38.6%) cases and scanty in 86 (61.4%) cases. Fine or large cytoplasmic vacuoles were prominent in 129 (92.1%) cases (Table 2).

Endocrine tumours. Three pancreatic endocrine tumours were all hypercellular and two were insulinomas by clinical presentation and by immunohistochemical studies. The third one was an incidental finding and was non-functional. The majority of the tumour cells were loosely cohesive, forming aggregates of varying sizes and with abundant single cells in the background. The tumour cells were polygonal with round uniform nuclei showing occasional nuclear pleomorphism and binucleation or multi-nucleation. Chromatin was fine and evenly distributed with occasional small eosinophilic nucleoli. Eccentrically

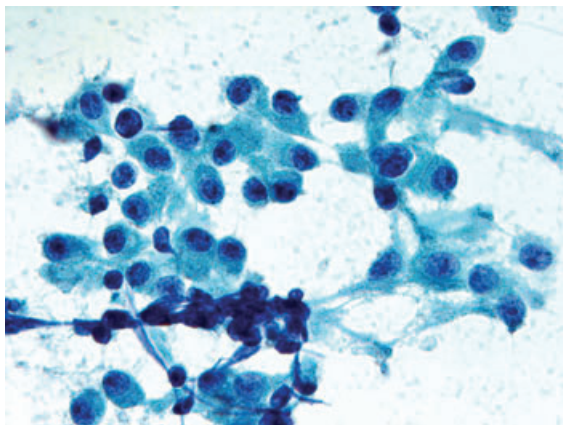


Figure 4. Loosely cohesive aggregates of plasmacytoid tumour cells with round nuclei and evenly distributed fine chromatin are features of most endocrine tumours of the pancreas (40×, modified Papanicolaou stain).

located nuclei (plasmacytoid appearance) were conspicuous features (Figure 4).

Malignant lymphoma. Two cases that were diagnosed as malignant lymphoma were both highly cellular and showed a proliferation of discohesive monotonous lymphoid cells with scanty cytoplasm, clumped coarse chromatin and a high N/C ratio. The monoclonality of B-cell phenotype was proved by immunohistochemistry performed on the cell blocks.

Solid pseudo-papillary tumour. This tumour, extremely cellular, was characterized by the presence of abundant papillae surrounded by multi-layers of small and uniform round to slightly oval cells associated with similar numerous discohesive cells in the background (Figure 5). The nuclear membranes were regular and smooth. Chromatin was fine and evenly distributed. Nucleoli were inconspicuous. The tumour cells exhibited a plasmacytoid appearance. Hyalinized collagen was seen. Immunohistochemical studies showed that the tumour cells were negative for keratin, chromogranin and synaptophysin. The diagnosis was confirmed by the surgical resection specimen.

Plasmacytoma. Plasmacytoma was cytologically identical to that seen in other locations. The smears were hypercellular and revealed various stages of plasma cells. Immunohistochemical studies, performed in the cell block, showed that the tumour cells were positive for lambda light chain and negative for kappa light chain.

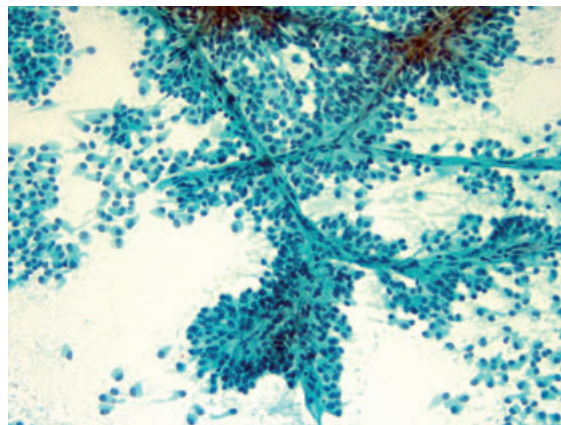


Figure 5. This solid pseudo-papillary tumour is characterized by abundant papillary fragments lined by multiple layers of small uniform round to oval tumour cells associated with numerous discohesive similar cells in the background. Notice the plasmacytoid appearance of the tumour cells (20×, modified Papanicolaou stain).

Normal elements from duodenum, stomach and pancreas. The presence of epithelial cells from the duodenum, stomach or pancreas was found in 77 positive/suspicious, three atypical, and 90 negative cases. In 13 positive/suspicious cases, the presence of contaminating epithelial cells and pancreatic acini made an accurate immediate evaluation extremely difficult (Figure 6) and the final diagnoses were made in cell block materials. Benign pancreatic acini and ductal epithelial cells were present in various numbers in all negative cases.

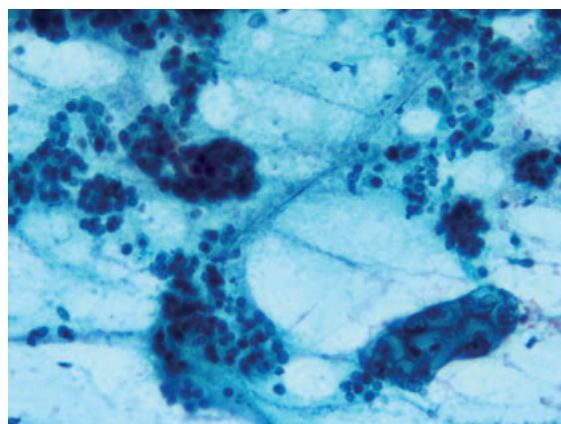


Figure 6. Numerous pancreatic acini abundant in another positive case and associated with malignant tumour cells in the right lower corner (40×, modified Papanicolaou stain).

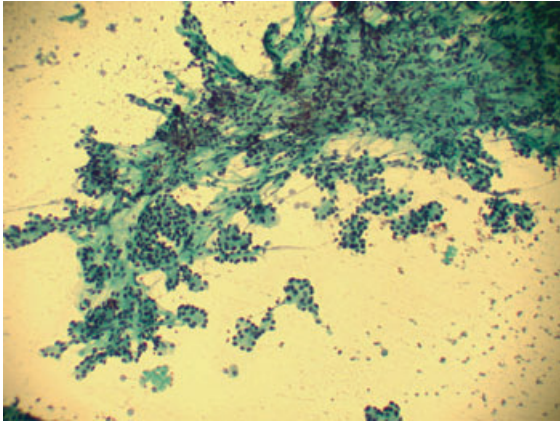


Figure 7. Chronic pancreatitis. Abundant benign pancreatic acini are present in this negative case and are associated with marked fibroblastic proliferation.

Chronic pancreatitis. Ninety-six patients had a negative cytological diagnosis, characterized by the presence of at least 5–10 groups of benign ductal epithelial cells or pancreatic acinar cells. Chronic inflammatory cells and fibrous tissue were occasionally seen (Figure 7).

Discussion

Cytological diagnosis of pancreatic neoplasms has always been challenging. The diagnostic criteria have been well established in specimens obtained by percutaneous CT, ultrasound^{17–26} and intraoperative approach.^{27,28} The current study focused on the diagnosis of adenocarcinoma, as it comprised 95.2% of the cases. Pancreatic adenocarcinoma has been previously reported to be associated with increased cellularity, predominantly one cell type, three-dimensional cell balls with overlapping pleomorphic nuclei, single cells, tall cells with large ‘tombstone’ nuclei, cells with high N/C ratio, irregular nuclear membranes, coarse and clumped chromatin, the presence of macronucleoli, mitotic figures and necrosis. In our study, cellularity was low in the majority of positive/suspicious cases. Compared with the percutaneous approach, EUS-guided FNA procures more cells more easily, assumably because of better visualization of the lesion and closer proximity of the needle to the lesion.^{14,15,29} However, the cellularity of any FNA specimen is always influenced by the technical skills of the person who performs the procedure and the physical features of the lesion being aspirated. For cellular lesions, such as endocrine tumour, malignant

lymphoma, solid pseudo-papillary tumour and plasmacytoma, high cellular samples were easily obtained after an average of two aspiration passes (range 1–3). By contrast, pancreatic adenocarcinoma is typically associated with a prominent desmoplastic host response and adjacent chronic pancreatitis. This will make a successful aspiration difficult. Sometimes even after multiple passes the cellular yields were still low. In cellular samples when pleomorphic nuclei, macronucleoli and necrosis are present, it is not difficult to make a definitive diagnosis. Our experience with low cellularity smears indicated that atypicality of the tumour cells was more important. For example, the presence of single cells, abnormal arrangement of sheets or clusters of tumour cells such as loss of polarity and the presence of three-dimensional balls, enlarged nuclei with prominent nucleoli and the presence of necrosis were important features. These features may be subtle and sometimes only a few were present in one case. Two patterns of necrosis were recognized. One was diffuse, when the entire background showed tumour diathesis, in which case conspicuous malignant cells of varying numbers were almost always present. Another pattern was that tumour diathesis was only focally present in association with a few single cells that were either slightly atypical or frankly malignant. The second pattern of necrosis was usually encountered in the low cellularity smears.

While the diagnostic criteria for pancreatic neoplasms are not significantly altered in specimens obtained by EUS-guided FNA, there are a few unique features associated with this technique. The most striking one, demonstrated in this study and recognized by others,^{14–16} was the presence of a variable number of normal duodenal or gastric epithelial cells and pancreatic acini in 52.4%, 30.0%, and 93.8% of positive/suspicious, atypical, and negative cases respectively. This was not unexpected as the pancreatic lesions were approached either transduodenally or transgastrically depending on the location of the lesions (head, body or tail). When the tumour was associated with pronounced pancreatitis, it was common to aspirate benign acini. Distinguishing normal from abnormal cellular elements was not always easy, as the presence of abundant normal cellular elements may either obscure the scanty malignant cells or mimic a malignant tumour. This was particularly troublesome when dealing with a well-differentiated adenocarcinoma. In these cases the presence of single cells and abnormal tumour cell arrangement were the

most consistent and reliable features. The presence of pancreatic acini in negative cases could also be problematic, as they were the cellular components of an aspiration in chronic pancreatitis. Therefore, one needs to be cautious in interpreting the significance of benign acini. Examination of the cell block may help make a definitive diagnosis. However, sampling error is unavoidable when the endoscopist cannot distinguish chronic pancreatitis from pancreatic carcinoma on ultrasound and only benign components are aspirated. In our experience, seven false-negative cases in the current study were such examples and the cell blocks in five of these cases contained only benign pancreatic acini. Therefore, although the positive predictive value was 100%, a negative diagnosis did not necessarily rule out malignancy (negative predictive value 82%).

The complementary importance of cell block in EUS-guided FNA has not been addressed before. Not only can cell block be used to confirm the cytological diagnosis of the smears and to perform ancillary studies when necessary, but it may also contain the only diagnostic material. In our study, the cell block included diagnostic material in 91 of 114 positive/suspicious cases. More importantly, it was exclusively diagnostic in 20% of these cases while the corresponding smears were non-diagnostic. The confirmation of endocrine tumour, malignant lymphoma and plasmacytoma was all based on the immunohistochemical stains performed on cell block materials.

Adequacy assessment is another debatable issue and no criteria have been suggested. Based on our experience with pancreas, there was no well-defined standard and it may vary slightly from case to case. If unequivocal malignant cells were identified, the specimen was satisfactory. When only blood, normal duodenal or gastric epithelial cells were seen, the specimen was unsatisfactory. For negative cases, at least five to ten groups of epithelial cells or pancreatic acini, 10 cells or more in each group, were required to be present in each slide in a minimum of three passes. To avoid false-negative diagnosis, it was crucial to communicate with the endoscopist for clinical presentation and ultrasonographic findings. When a preliminary interpretation was made, the three elements should correlate. There were occasions when the ultrasonographic findings were not typical for benign or malignant lesions and the endoscopist was uncertain about what he or she was dealing with. Sometimes the cytological interpretation did not correlate with the ultrasound findings. A comment in the report

would be important in these cases to recommend clinical follow-up or even surgical biopsy for confirmation.

Early studies have indicated that the presence of a cytopathologist improved the diagnostic accuracy dramatically.¹⁶ Immediate assessment of specimens has important educational, clinical and economic implications; it helps the endoscopist manipulate the needle more accurately, obviates unnecessary passes, decreases procedure time and therefore shortens the sedation period of patients. Moreover, it helps clinicians in their decision-making to label the malignant tumours for subsequent therapeutic intervention. However, this has not been a standard practice in medical centres where EUS-guided FNA is performed¹³ because of time, manpower and cost issues. Interestingly, Shin *et al.*¹⁵ reported that the inadequate specimen rate (13.2%) while no immediate assessment was provided was not markedly different from that of other studies wherein immediate assessment during the procedure was done.^{4,5,8,14} It has been recommended that three to six passes be performed when no pathologist is available for adequacy assessment at the time of the procedure.^{9,15} In our institution, specimen adequacy and preliminary interpretation of each pass are provided to the endoscopist in every patient during the procedure by a cytotechnologist and/or a cytopathologist. This intimate teamwork approach between the endoscopist and the cytopathologist has proved vital to the effective application of this new technology and may partly explain the significantly lower rate of unsatisfactory specimens (5.2%) in the current study.

In summary, EUS-guided FNA is both sensitive (94.6%), specific (100%) and accurate (95.6%) in the diagnosis of pancreatic neoplasms in both low and high cellularity samples in combination with cell block materials. The assessment of specimen adequacy depends upon the cellularity and ultrasonographic features of the lesion aspirated. Recognizing the contamination of epithelial cells from GI tract and pancreas is important to avoid misinterpretation. Finally, close collaboration between the endoscopist and the cytopathologist is pivotal in the success of this diagnostic procedure.

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