MY APPROACH

My approach to pancreatic fine needle aspiration

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Pancreatic fine needle aspiration cytopathology has earned a reputation as a rapid, safe, accurate and cost-beneficial modality of investigation of pancreatic mass lesion. Optimal results can be expected only if these procedures are undertaken regularly in gastroenterology departments processing large numbers of patients; occasional aspirators cannot exploit the technique to full potential. Professional teams following a dedicated approach to patient selection and management develop requisite expertise over time. Cytodiagnosis rests as much on morphological examination as on the milieu in which it is practised, and as much on sample quality as on cellular criteria. This article focuses attention on specimen handling, with particular reference to rapid on-site evaluation. The significance of particular cytodiagnoses in patient care is evaluated, and tumour types that may be encountered are enumerated and illustrated.

The brief of this article is not to summarise the excellent chapters on pancreatic fine needle aspiration (FNA) cytopathology found in recent texts,^{1–6} nor to emulate excellent literature reviews on the topic,^{7–12} but rather to offer an approach to the procedure developed over 25 years. First at the University of the Witwatersrand, Johannesburg, South Africa, and later at the University of Vermont, Burlington, Vermont, USA, it has been my privilege to be associated with this extraordinary technique. I have seen it evolve from its origins in the “dark ages” of pancreatic exploration before the advent of imaging techniques such as ultrasound and computed axial tomography, right through to the utilisation of endoscopic ultrasound-guided FNA (EUS-FNA) technology. This involved working in frozen section rooms on specimens aspirated intraoperatively,^{13–15} in radiology departments on percutaneous samples obtained by imaging,^{16–20} and most recently, in endoscopy suites, on material acquired transduodenally by EUS.^{21–30} Each approach seemed revolutionary on introduction, each yielded excellent results if the operator was experienced, and all pre-empted the potential and real complications of pancreatic core or wedge biopsies—that is, fistulas, haemorrhage and tumour spillage. To properly fulfil the promise of pancreatic FNA, both the operator who procures the sample and the pathologist who interprets it bear equal responsibility in commitment to the technique and in acquiring diagnostic skill, which may take many cases, many circumstances and also many years to achieve.

CLINICAL CONSIDERATIONS

Indications, patient parameters and follow-up

The presence of a mass lesion, cystic or solid, is the only genuine indication for pancreatic FNA, although some use a “scouting” technique to sample multiple sites in an enlarged organ. The need to aspirate and the approach (endoscopic, percutaneous or operative) will have been decided before the pathologist becomes involved. The pathologist needs to know a concise clinical history including age, gender, symptoms, physical findings, radiological features, prior tumours and prior treatment, all of which may bear on the diagnosis. A clinical working diagnosis is helpful, but not absolute. For example, a history of alcohol misuse, recurrent upper abdominal pain and recent onset of diabetes may suggest chronic pancreatitis, but would not entirely exclude the development of carcinoma within this background. Conversely, onset of painless jaundice in a patient with weight loss will instantly raise the likelihood of carcinoma, again not with complete specificity. I regard four parameters as critical to the evaluation of the cell sample, and will not proceed without them: age, location in the pancreas, solid/cystic nature of the mass and whether a prior biliary stent has been placed.

Clinical concerns do not cease on completion of the procedure. Some patients have surgical excision, during which histological correlation is obtained. Others, however, will have only palliation after a malignant cytological report, and no tissue confirmation. It is obligatory to follow their progress to determine whether clinical outcomes are compatible with diagnoses rendered. If the patient is alive and well a year after a diagnosis of malignancy without surgery, the cytological evaluation should be reviewed very carefully!

The operator

Mutual understanding must exist between the aspirator and the pathologist, which, if they collaborate for any length of time, will develop into implicit trust, with better patient care as the outcome. At the Johannesburg Hospital (Johannesburg, South Africa), most operable cases were handled by Professor JA Myburgh, Professor of Surgery. It was he who instituted pancreatic FNA, first in his unit, then in the many other surgical units of the Medical School, University of the Witwatersrand, and soon by osmosis into the private surgical practices of the greater Johannesburg area. These cases were done

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Abbreviations: EUS, endoscopic ultrasound; FNA, fine needle aspiration; IPMT, intraductal papillary mucinous tumour; MCT, mucinous cystic tumour

intraoperatively at the time of biliary bypass surgery, before the advent of EUS-FNA procedures or stent placements. Professor Myburgh's preference for the intraoperative route stemmed from the work of Warshaw and colleagues in Boston,³¹ which showed a higher incidence of malignant cells in peritoneal washings taken at the time of pancreatic surgery from patients who had undergone prior radiologically guided FNA compared with those patients who had not had prior FNA. The potential for peritoneal spread of otherwise operative lesions prompted Professor Myburgh to popularise the operative transduodenal route, during which peritoneal surfaces are not violated. Intraoperative cytopathology was therefore required, with all the stress to the pathologist of instant diagnosis. Table 1 indicates that Professor Myburgh's unit achieved a sensitivity of 95.2% for neoplasia in 128 cases, compared with other intraoperative procedures done at several other academic hospitals by a variety of surgeons, who achieved a rate of 88.8% for neoplasia in 137 cases; multiple radiologists, aspirating generally larger, more advanced tumours, achieved adequacy rates in the region of 85.4% for 195 cases. The table also indicates that sensitivity was closely related to adequacy, being lowest in the radiology cases at 83.6%, and highest for the professorial surgical cases at 99.2%.

In the past 5 years, I have worked with Richard Zubarik, MD, Associate Professor of Medicine, who performs EUS-FNA at the Fletcher Allen Health Care, University of Vermont. Once again, understanding of the advantages and limitations of the endoscopic approach, as well as the capabilities of on-site cytological evaluation, has developed. Adequacy rates for the investigations in this facility are in the region of 88%, with one operator doing the procedures (internal cytopathology department audit). For best outcomes, procedures should be performed in centres of proven upper gastrointestinal excellence, both investigational (radiology, endoscopy) and therapeutic. This statement will appear unfortunately elitist, but the technique is challenging and, if it is to fulfil its best potential, these requirements are necessary.

The (cyto)pathologist

I do not believe that one can be a reliable, occasional interpreter of pancreatic cytomorphology. Frequency and regularity of exposure to pancreatic cell samples are critical to development of expertise. I am an unshakable proponent of interpretation by the so-called "ROSE" (rapid on-site evaluation) technique. This implies that pancreatic FNA is best handled by the staff of a laboratory of medium to large size, from which pathologists, with or without registrars or accompanying cytotechnologists, can be spared whenever required for procedures; pathologists cannot be distracted by concerns of routine work accumulating in their absence. FNA duration may be equal to that of the

frozen section; on average, however, with easy access and good aspirators, the cytological component can be completed in 15 min.

THE PROCEDURE

Cyst fluid aspirates

Fluid aspirates originating from pancreatic cyst are easily interpretable by cytological means, but require processing in the laboratory after centrifugation. This is not a suitable technique for on-site evaluation. A cyst aspiration should be as complete as possible, with the resulting fluid sample submitted quickly to the laboratory. In emergency situations, if the laboratory is forewarned, an interim result can probably be available in 45–60 min.^{32–34}

Solid lesion, on-site evaluation

Evaluation of samples obtained by FNA during the procedure is possible for solid tissue aspirates, and the on-site assessment should be as confident and accurate as the sample permits. Time spent by the patient in the operating room, endoscopy suite or radiology department is expensive. The duration of the investigation is generally determined by the number of needle passes, which in turn depends on sample adequacy. An experienced operator will require no more than 2–3 needle passes to appropriately sample a target lesion. On-site evaluation ensures, in most cases, that the patient will not be required to return for a second purely diagnostic interventional exercise.

Slide preparation

Cytopathologists and cytotechnologists possess an instinctive or learned ability to prepare cells for accurate microscopy. Many clinicians may acquire abundant samples, but spoil the material by suboptimal spreading technique. On site, it is the function of the pathologist or technologist, not the operator, to spread the slides. The amount of blood present determines the quality of the cell spread, whether they will be of textbook quality, or diluted, clumped or trapped in clot. To this end, the thinner the needle, the better for optimal cellular display. We insist that a fine needle is used (a 21-gauge or thinner needle), and prefer to work with material obtained by 23 or 25 gauge needles. Many pathologists make multiple slides with each needle pass, to ensure that they have spare slides for special stains. My preference is to limit the cell sample to fewer slides, as (1) this means I am not rushed in assessing too many slides on site, (2) in the pancreas, immunochemistry does not play as large a role as it does in other sites such as lung or liver and (3) I feel that architecture is better maintained if I do not spread the cells using too much pressure. Obviously, I aim for a monolayer as far as is possible.

Table 1 Sensitivity for malignancy in pancreatic fine needle aspiration: Johannesburg Hospital Experience, 460 cases*

Modality of approach	Cases (n)	Tumours (n)	Adequacy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Radiological cases	195	123	83.6	85.4	100	100	80.0
Surgical cases (total all units)	265	185	95.8	92.4	96.3	98.3	87.6
Professorial unit	128	105	99.2	95.2	95.7	99.0	82.0
All other units	137	80	92.7	88.8	96.5	97.3	92.0

NPV, negative predictive value; PPV, positive predictive value.

*Data presented at the 22nd European Congress of Cytology, Amsterdam, The Netherlands, 25–28 September 1994.

Staining

Cytopathologists should work on-site with the rapid stain with which they feel most confident. My chief experience has been with the rapid (4 min) Papanicolaou stain. Its great advantages are that morphological features are well known to the cytopathologist, the rapid stain is permanent and the slide will not require destaining/restaining in the laboratory. Many use the air-dried Romanowsky or Giemsa stains on air-dried material for on-site evaluation. If they feel confident with this, they should not change. However, I personally feel that air-dried samples are not optimal for rapid, easy on-site assessment of difficult pancreatic lesions; nuclear detail is of paramount importance in the interpretation of pancreatic cytology, and Romanowsky stains are known not to preserve nuclear morphology well. More recently, I have used the on-site Toluidine Blue stain, which is also a superb nuclear stain; it has the advantages of being ultra-fast, taking only a few seconds, and of not overstaining the blood. However, the slides require later destaining and restaining with a permanent stain in the laboratory, and this may dislodge cells or cause the permanent stain to be inferior. Haematoxylin and eosin staining in cytopathology is strongly discouraged, but those familiar with it from frozen sections may find it adequate.

Triage for ancillary studies

On-site evaluation permits the collection of material into appropriate alternate media or fixatives, if required. As alluded to above, immunochemistry is not required as often for pancreatic FNA as in other sites. Nevertheless, for neuroendocrine tumours, it is appropriate to make further slides for confirmatory stains.³⁵⁻³⁶ To this end, it has also been our practice to rinse each needle pass into CytoLyt (Cytoc, Boxborough, Massachusetts, USA), to save all aspirated cell groupings for immunostudies on thin-layer preparations.³⁷ Should an aspirate appear inflammatory, which is unusual in the pancreas, culture media can be injected with the aspirate at this point. If the aspirator has sampled a lymphoid population from adjacent nodes or the pancreatic parenchyma, this is the opportunity to collect further needle passes into RPMI for flow cytometry. In rare cases, perhaps for teaching purposes, it may be appropriate to do electron microscopy, placing material for this purpose into glutaraldehyde. As with immunochemistry, this is seldom needed in the pancreas.

CYTODIAGNOSIS

The major decision on-site is whether the lesion is benign or malignant. This very black-or-white requirement is nowhere more essential than intraoperatively, when a Whipple's pancreatotomy may hinge on cytodiagnosis. This is major surgery with intraoperative, as well as later, morbidity and mortality. With the advent of preoperative endoscopic evaluations, pressure to provide instant benign or malignant diagnoses has diminished considerably, the effort being to procure sufficient material, appropriately fixed, for instant or deferred definitive diagnosis, the latter after less stressed microscopic evaluation, intradepartmental consultation and immunochemistry, if required.

Pancreatic versus contaminant cells

Whatever the approach chosen, organs other than the pancreas are traversed, albeit only the duodenal wall in endoscopic and surgical cases. The importance of distinguishing benign or hyperplastic duodenal or ampullary lining epithelia from pancreatic parenchymal epithelium is obvious. Pancreatic parenchyma itself may yield material other than ductal components, mainly three-dimensional groupings of acinar cells, which, to the unwary, may appear as rounded structures

suggestive of neuroendocrine tumours or adenocarcinomas (figs 1, 2).

My approach is to skim rapidly over the entire slide at low magnification to assess cellularity and architecture. In a good aspirate, groups from pancreatic ductal carcinoma will immediately manifest as different from any normal components that may be present. It is at this very early point that a diagnosis can be rendered, literally within seconds of putting the slide under the microscope, but this can be accomplished only if the FNA is of superior quality and multiple abnormal groupings are present. Cytopathologists function by pattern recognition. It is erroneous to imagine that a finely detailed textbook-type assessment of morphology is mentally applied when looking at a good aspirate. With moderately or poorly differentiated tumours, as the material comes into focus, so it triggers a "malignant" response from the viewer. Challenges may arise when the tumour is well differentiated. It is my practice to look for the so-called "drunken honeycomb" appearance of crowded epithelial sheets, as well as to note nuclear size and pleomorphism. Crowding within sheets to the extent that nuclei appear squared off due to internuclear crowding rather than round immediately suggests malignancy. In pancreatic ductal carcinoma, the criteria which I find of greatest value on-site are nuclear outline irregularities, notches, grooves and indentations, as well as the margination of chromatin to the nuclear envelope, with resultant clearing or pallor of chromatin.

This appearance is not specific to the pancreas, but certainly assists in recognition of better-differentiated tumours (fig 2). In general, I follow the guidelines given in the literature, and particularly those from the MD Anderson Cancer Center, Houston, Texas, USA, by Lin and Staerckel on the criteria for very well differentiated adenocarcinoma of the pancreas.³⁸⁻⁴³

Mucinous tumours

The elucidation of mucinous tumours of the pancreas has evolved considerably over the past 10 years. Ductal adenocarcinoma of mucinous type, sometimes called colloid carcinoma, is relatively rare, and carries the same poor prognosis as other pancreatic cancers. Similarly, ductal adenocarcinoma of signet-ring type is rare, readily recognisable to the cytopathologist and of limited prognosis. Two other mucinous tumours are encountered more often; these are intraductal papillary mucinous tumour (IPMT)⁴²⁻⁴⁷ and mucinous cystic tumour (MCT).⁴⁸⁻⁵² The clinical difference between these is greater than their cytological difference, an important reason to be familiar with the age and gender of the patient, as well as the nature and site of the lesion. The classic MCT is an indolent tumour of

Figure 1 Sheet normal pancreatic duct cells. Flat monolayer honeycomb, small group of four cells seen in profile with tall columnar appearance, monotonous even round nuclei and absence of goblet cells. (Papanicolaou $\times 40$).

Figure 2 Aggregates of pancreatic ductal carcinoma. Irregular or "drunken" honeycomb appearance of enlarged, crowded ductal cells. Note increased nucleocytoplasmic ratios, margination of chromatin to inner nuclear envelope and pale chromatin. (Papanicolaou $\times 40$).

middle-aged to elderly women, occurring more often in the distal than in the proximal pancreas. Imaging outlines a very well-circumscribed round tumour, without connection to the pancreatic duct, from which thick viscous, glistening material is aspirated. Microscopy shows intracellular and abundant extracellular mucin, regularly honeycombed epithelial sheets and, under circumstances of malignant transformation, very classic features of nuclear malignancy. IPMT has only recently been characterised cytologically. Once again, radiological and clinical input is essential, as this tumour forms abundant mucin within dilated pancreatic ducts, to the extent that mucus may be seen flowing from a patulous ampulla by the endoscopist. Naked-eye evaluation of the aspirate will again show abundant, glistening, clear material that spreads easily. Microscopy demonstrates a background of mucin, easily seen as fibrillar in nature, with parallel strands visible at high-power microscopy.

This contains cell islands and fragments, some of which are rounded and papillary, comprising mucin-producing and mucin-containing cells (fig 3). As with MCT, IPMT has a better prognosis than the usual pancreatic cancer (fig 4). However, it does have a potential for malignant transformation, and when this occurs, cells showing the true nuclear criteria of malignancy will be apparent.

Figure 3 Mucinous tumour, low power view. Cellular aspirate demonstrating abundant background mucin. Clusters of cells are embedded within the mucus. This could be from a mucinous cystic tumour or from intraductal papillary mucinous tumour. (Papanicolaou $\times 10$).

Figure 4 Mucinous cystic neoplasm, oil immersion view. A sheet of mucin producing columnar epithelial cells seen at very high magnification, demonstrating pale intra-cytoplasmic secretion, which is mucin. (Papanicolaou $\times 40$).

Abundant mucin is a "gift" to the cytopathologist. Both MCT and IPMT are tumours that have a benign origin, a long intraepithelial course and ultimate predisposition for malignant transformation. Unless obviously inoperable, both require surgical excision, with examination of multiple blocks to determine whether or not malignant conversion has occurred. If mucin is abundant in an aspirate, and one is certain that the mucin is associated not with duodenal epithelium but rather with neoplastic pancreatic ductal elements, the final assessment of that epithelium must be histopathological, and thereby becomes non-problematic on-site. The appropriate cytodiagnosis is that of mucinous tumour (either mucinous cystic or intraductal, depending on the radiological features). The presence or absence of invasive carcinoma is deferred to subsequent surgery and histopathological evaluation; if obvious malignant cells are seen, however, these must definitely be reported cytologically.

Uncommon tumour types

Variants of pancreatic ductal carcinoma other than the mucinous variants are readily recognised cytologically. For example, it is known that osteoclastic giant cells, pleomorphic giant cells, or both, may be seen in pancreatic tumours.⁵³⁻⁵⁹ In addition, adenosquamous variants⁶⁰⁻⁶² and anaplastic variants

Figure 5 Neuro-endocrine tumour. This tightly packed cell cluster demonstrates one FNA pattern of neuro-endocrine or islet cell tumour. The cells are crowded in three-dimensional acini, and are characterized by granular chromatin. (Papanicolaou $\times 40$).

Figure 6 Solid-cystic pseudopapillary neoplasm, fixed. On the fixed slide, the stromal tissue forming the core centres is virtually inapparent, giving a false impression of acinar formations. This may mimic islet cell tumour, but displays bland chromatin. (Papanicolaou $\times 40$).

may be encountered and readily recognised. Appearances of all variants are known to cytopathologists from other sites

Islet-cell tumours are encountered in FNA cytology and are familiar to the cytopathologist on the basis of architecture and nuclear features.^{63–69} These tumours may be highly vascular, the cells intensely three-dimensional, or they may be displayed as a single-cell population. The cells are of small to moderate size, with relatively low nucleocytoplasmic ratios, abundant pink cytoplasm, and classic nuclei containing the so-called salt and pepper chromatin granularity (fig 5). Nucleoli are not obvious features of these tumours. Architecture may also include cell ribbons, acinar formations and even papillary formations. Confirmation by synaptophysin or chromogranin-A immunostaining is obtained subsequently. The surgical approach to neuroendocrine tumours may be different from that of ductal adenocarcinomas, as the prognosis too is different; this makes rapid on-site diagnosis essential.

Solid and cystic papillary neoplasms of young women form a distinct clinicopathological entity. The best prompt to diagnosis is the unusually young age of female patients. The cytopathology of these tumours has been characterised in many publications,^{70–72} but essentially includes cellular smears, containing pseudopapillary formations (ie, structures with cores lined by ragged epithelium, without the smooth cytoplasmic outlines of true papillae). The nuclei themselves are generally

Figure 7 Solid-cystic pseudopapillary neoplasm, air-dried. Air drying, with Romanowsky-type staining is required to appreciate the inner stromal vascular core, characteristic of the pseudopapillae of this tumour of young women. (Diff-Quik $\times 40$).

Figure 8 Lymphoma, low magnification. Monotonous population of lymphoid cells, seen at low magnification, from a follicular low-grade lymphoma of peri-pancreatic nodes, in a patient presenting with obstructive jaundice and irregular "pancreatic" mass. (Papanicolaou $\times 40$).

rather bland, and lack the grainy chromatin of neuroendocrine tumours, which they resemble in terms of size. An essential observation is the presence of stromal cores, more easily seen on air-dried slides, but visible on Papanicolaou stains, if looked for (figs 6 and 7).

This is an area in which numerous lymph nodes are present, and it is possible to encounter primary pancreatic lymphoma or nodal lymphomas in the region. One should recognise the characteristic single-cell appearance of these lymphoproliferative neoplasms,^{73–76} and be prepared to immerse further needle passes into RPMI for flow cytometric evaluation. This is more comprehensive than immunostaining for B cell and T cell markers. If the triage has not included cells in RPMI, basic phenotyping can be performed on cells by immunocytochemistry (figs 8 and 9).

Very rare pancreatic tumours

Too few cases of serous cystadenoma have been described to enable confident cytological assessment.^{77–78} The only case I have encountered in 25 years was missed cytologically three times! A middle-aged male patient was referred three times over 18 months for aspiration of a stable lesion, which the third radiologist finally interpreted as showing the radiological microcysts diagnostic of this tumour. Even on review, the

Figure 9 Lymphoma, high magnification. Dispersed malignant lymphoid cells from a diffuse large B-cell non-Hodgkin's lymphoma, presenting with upper abdominal symptoms, and diagnosed by US-guided transabdominal FNA. (Papanicolaou $\times 100$).

FNAs were very sparsely cellular, containing only non-diagnostic rare small bland cells; all were reported as “inadequate”. Had the initial radiologist mentioned the multicystic nature of the lesion, it might have been possible to call the watery cytology sample benign, and therefore consistent with the clinical and radiological presentation, rather than inadequate.

I have not seen a proven case of acinar cell carcinoma cytologically. From the few reports which exist, it appears that this is a rather small-celled neoplasm, easily denuded of cytoplasm in the aspiration process, which enters into the differential diagnosis of neuroendocrine tumours, with clumped chromatin, and also very prominent nucleoli.^{79–81}

Acute haemorrhagic pancreatitis is seldom referred for FNA, as the clinical presentation is that of an acute severe illness in a patient who might previously have had similar episodes. If FNA is performed, the sample will be characterised by numerous acute inflammatory cells, admixed with duct epithelial cells in various stages of degeneration and repair. Regeneration may be profuse and may mimic malignancy. It is necessary to obtain several needle passes proximal and distal to the initial FNA to ensure that the inflammation is not secondary to obstruction by a small carcinoma. Chronic pancreatitis is more likely to enter the realm of cytopathology, as symptoms can mimic pancreatic cancer. Once again, epithelial proliferation may be a prominent component; it may be sufficiently severe to warrant consideration of pancreatic carcinoma. It is essential to get multiple needle passes to visualise sufficient numbers of cells to ensure that classic features of malignancy are not present. Particularly in the early days of pancreatic FNA cytology, chronic pancreatitis was always advanced as the lesion most likely to lead to false positive diagnoses of malignancy.^{82–84}

COMPLICATIONS

To date, to my knowledge, there have been six deaths reported after pancreatic FNA. Five were ascribed to pancreatitis, and one to sepsis after needling of a pseudocyst. Morbidity from severe but non-fatal pancreatitis has been described in up to 3% of patients. Apparently, none were patients harbouring pancreatic carcinoma. All were aspirated because their inflammatory lesions were thought to resemble carcinoma. Aware of this, some clinicians hesitate to aspirate suspected cases of pancreatitis unless there is clear evidence of nodule formation. Patients are monitored after pancreatic FNA by pulse rate and blood pressure readings, and for signs of abdominal pain or increased temperature. There has been no reported death from haemorrhage after such a procedure, as far as I am aware.^{85–86}

Tumour seeding of the needle track is always raised as an alarming issue. In an early review of abdominal FNAs with needle track spread, Roussel *et al*⁸⁷ evaluated seven cases; they concluded that at least three centres did not use a true fine needle, having used needles of 16–18 gauge. A more recent literature review by Smith⁸⁵ identified 11 further instances of needle track spread, five of which used needles >21 gauge, which were thus not “fine”. Smith calculated the incidence of seeding as 4.5 per 100 000 transabdominal FNAs. It would appear that cutaneous needle track seeding is an extremely rare event; with the trend to endoscopic procedures, it is hoped that this unusual event will become even more rare.

Transperitoneal, rather than needle track, spread may be of greater concern. Subsequent to the work of Warshaw, however, two later prospective studies of peritoneal washings in patients undergoing pancreatic surgery with or without prior FNA have been published.^{88–89} The studies concluded that positive peritoneal fluid was likely a consequence of high-grade, high-stage cases of poor operability, rather than a cause of unresectability, early metastasis and short survival. Peritoneal

fluid positivity was not seen in early tumours, and no survival disadvantage was shown when this did occur. Once again, the entire concept of peritoneal spread after FNA may become historic, now that endoscopic approaches are used.

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