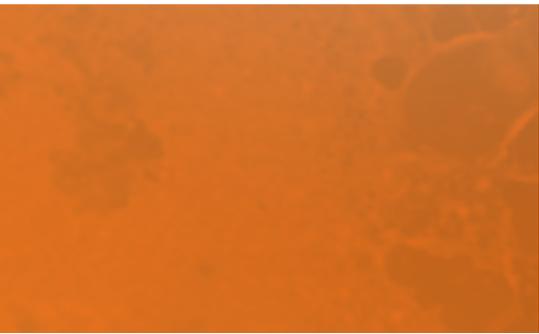


What is the Desired Algorithm for the Utility of a Cell Block?

ACQUISITION OF TISSUE FOR CELL BLOCK AS A WAY TO
INCREASE DIAGNOSTIC SENSITIVITY AND SPECIFICITY



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Why Block the Cell Block?

Despite the potential diagnostic utility of the cell block in Fine Needle Aspiration (FNA), it appears to be underutilized in the aspiration of lesions performed by Endoscopic Ultrasound (EUS). Several factors may contribute to the infrequent use of the cell block as a diagnostic tool. Among these are the perceptions that the cell block cannot contribute any additional pertinent information, it invokes additional unnecessary costs, lengthens the procedure, or provides insufficient tissue for further ancillary testing. The majority of these sentiments do not hold up under close scrutiny. Acquisition of tissue for cell block can increase both diagnostic sensitivity and specificity (through both cellular morphology and ancillary testing). It requires minimal effort and is extremely cost efficient. Moreover, tissue preserved in cell block can be readily shared for second opinions and research without fear of losing the original diagnostic smear specimen. (see p.2 Algorithm Showing the Utility of a Cell Block or Tissue Core.) For these reasons, the question of why the cell block is not routinely used for EUS-FNA procedures must be addressed.

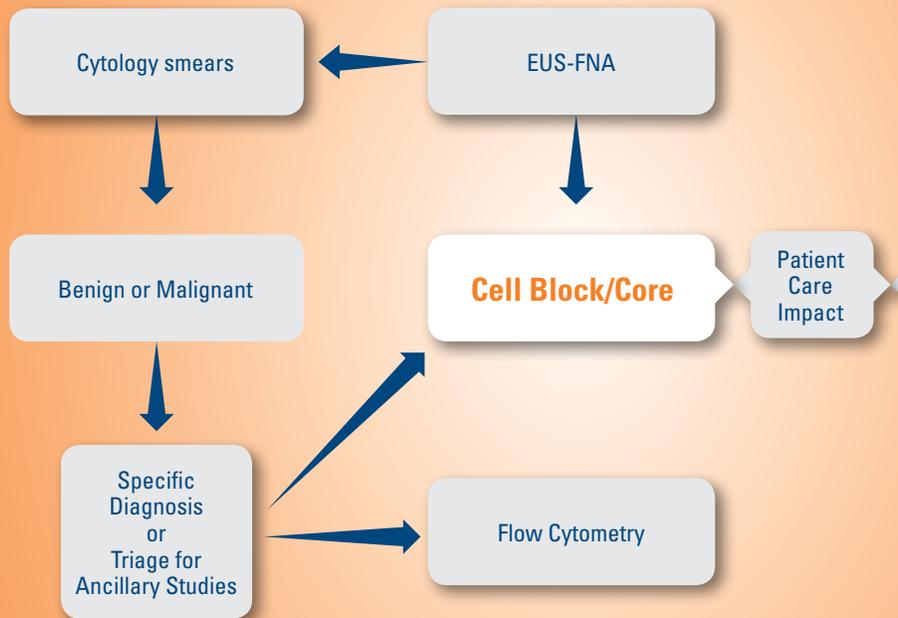
Immediate Gratification

Often, patients present for EUS-FNA after the clinical and/or radiologic work up suggests a mass lesion may be present. Specifically, EUS-FNA is useful in small, deep-seated lesions which may not be apparent by CT or are difficult to reach by the transcutaneous approach. The immediate questions posed are: Does the patient have a malignancy? If so, can it be resected and what is the tumor stage? These specific questions can typically be answered by the endoscopist and pathologist without the use of a cell block. The endoscopist is usually content with localizing and acquiring diagnostic material while the pathologist is pleased to have been able to confirm the clinical suspicion. All appears well.

However, imagine if two days later the pathologist receives a call from the clinician stating the patient has a history of lung carcinoma. He notes that the cancer was resected at another institution, and wants to know if this malignancy in the pancreas is of pancreatic origin or a metastasis from the lung. Or he may want to know if a poorly differentiated

Algorithm Showing the Utility of a Cell Block or Tissue Core

More is Better: Tissue is the Issue



Can Increase Diagnostic Yield/
Accuracy When ROSE is not Available

Can Yield More Material for
Differential Diagnosis

Can Be Used for
Molecular Testing

Can Be Used for IHC Stains
for Confirmatory Testing

Can Yield Histological (Tissue)
Architecture not Present on Smears

Can Be Used for Future
Molecular Targets

Can Be Used for Second
Opinion or Tissue Recuts

Can Be Used for Patient Care
Oriented Research

Can Be Used for Special
Stains for Organisms

Timeline for Pancreatic Cancer

History

- Survival rates for lung, pancreatic and gastric cancer have remained stable for 40 years
- Pancreatic CA has the lowest survival rate of any solid cancer
- Ca-19 was once billed as an accurate tumor marker for pancreatic CA

Present

- 80% of patients undergoing a Whipple procedure will die within 5 years
- EUS-FNA is now commonly used to diagnosis pancreatic cancer
- Histology is now recommended by ESGE in addition to cytology for the diagnosis of pancreatic cancer
- The NACB-(National Academy of Clinical Biochemistry) as well as the EGTM (European Group on Tumor Markers) no longer support the routine use of CA-19-9 for the diagnosis of pancreatic cancer
- Early diagnosis of pancreatic cancer has the potential to save lives

Future (evolving now)

- Breast cancer has had a 24% reduction in deaths from 1990-2005
- This can at least partially be attributed to the identification of specific molecular targets (HER-2)
- These targets are now being identified in gastric and esophageal adenocarcinoma
- Identification of the molecular targets require sufficient tissue in order to perform IHC staining, in situ hybridization or PCR
- Once a specific molecular aberration is detected the patient can receive targeted chemotherapy which has been shown to increase survival

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tumor could be further characterized to help guide clinical management? Unfortunately in these cases, the pathologist will have to say that no material was collected for cell block so no further diagnostic testing can be performed. This may lead to a repeat EUS-FNA procedure or a more invasive procedure (i.e., biopsy) to acquire additional tissue. The patient has to undergo additional unnecessary procedures, delaying a diagnosis, increasing health care expenditures and possibly morbidity. This is an unfortunate consequence of not procuring sufficient diagnostic material for ancillary studies at the time of the initial procedure.

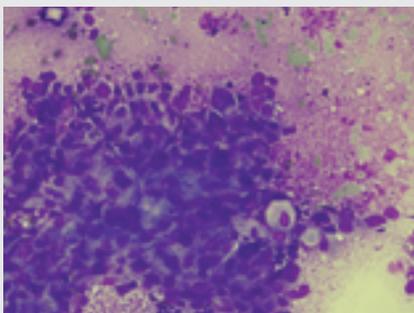
Back to the Future

As a practicing pathologist, it is imperative to have a good rapport with your endoscopist. As a team, we must evaluate our errors, be it sampling or interpretative, to determine what could have been done differently in order to obtain the correct diagnosis or convey the diagnosis in a more timely fashion. Through our ten-year institutional experience with EUS-FNA we have come to recognize that a good cell block can often help distinguish between disparate entities including: chronic pancreatitis, pancreatic adenocarcinoma, GIST, schwannomas, melanomas, non small-cell carcinoma, and many metastatic lesions. The high diagnostic accuracy of EUS-FNA often obviates the need for further diagnostic work up. This is especially true when the question is limited to whether the lesion is benign or malignant and determination of the primary differentiation (i.e., carcinoma vs. spindle cell lesion). However, in the age of targeted therapy, it is not sufficient to state that a lesion is a poorly differentiated malignancy. This is true even if the tumor is a stage IV unresectable cancer. For example, if a patient is found to have an unresectable submucosal spindle cell lesion, confirmed by immunohistochemical analysis to be a c-KIT positive GIST, treatment with targeted adjuvant therapy (Gleevec) may significantly reduce the tumor bur-

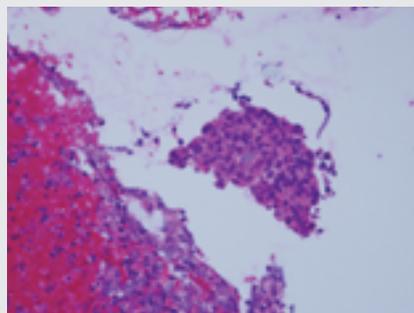
den, decrease the morbidity and possibly lengthen survival. To ensure that every patient is getting the most specific diagnosis, we routinely perform cell blocks on EUS-FNA specimens. Our endoscopists often alert us during the procedure if they suspect a potential metastasis. Alternatively, as pathologists we often raise the possibility of an occult primary if atypical features are present. We also communicate with the endoscopists if a lymphoma is suspected, or if granulomatous lesions are present so that material can be collected for cell block, flow cytometry and cultures, respectively. Of note, flow cytometry requires approximately 20,000 cells per sample (depending on the analyzer). As such, the quantity may not be sufficient for diagnosis by flow but very often the diagnosis can still be made with a cell block. Additionally, flow cytometry is not useful in the diagnosis of Hodgkin Lymphoma. Fortunately, the diagnosis can often be confirmed by CD15 and CD30 positivity in the cell block tissue.

Collection and Processing

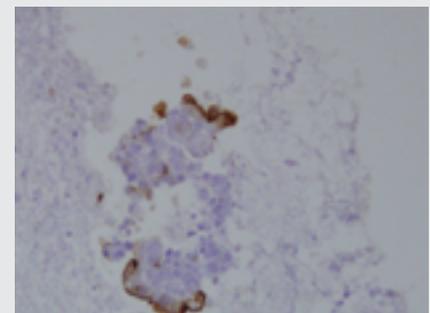
Acquiring additional tissue for cell block is not technically difficult. Needles can be flushed with air or rinsed with a saline solution after smears have been prepared to express any residual cellular material. Two additional dedicated passes, solely for cell block preparation is suggested to increase the likelihood of obtaining sufficient diagnostic tissue. The residual cellular material within the needle is expressed or flushed into a cell preservation solution. The vial containing loose cells and small tissue fragments is taken to the laboratory and centrifuged. The cell button is subsequently combined with plasmin and thrombin to form a clot (other methods for cell block preparation are widely available as well, i.e., agar gel, sediment or Cellient automated cell block processing). The clotted solid tissue is then processed like other histological specimens, i.e., paraffin embedding, hematoxylin and eosin staining.



Patient with known history of ovarian cancer, three years post chemotherapy and resection. EUS-FNA of pancreatic mass showed adenocarcinoma.



Cell block showing malignant tissue fragment.



Cell block staining for CA19-9 and CA125 were performed. The CA 19-9 was negative and CA125 (shown) was positive, supporting the diagnosis of metastatic ovarian carcinoma not a pancreatic primary.

Saved by the Cell

At this point you may be thinking, “Great. If I am worried about a metastasis or I anticipate needing molecular studies the cell block is great, but what’s the value of a cell block in diagnosing ‘usual cases’ of pancreatic adenocarcinoma?” “Can’t most pancreatic adenocarcinomas be easily diagnosed on conventional cytology smears?” The answer to this very logical question is an unequivocal yes. MOST pancreatic adenocarcinomas can be diagnosed only on the basis of the cytology smears, but is MOST good enough?

The value of the cell block in such cases can be illustrated by two recent examples at our institution. In both of the following situations the cell block was pivotal in both characterizing the pancreatic lesion and distinguishing it from other malignancies.

The first case was a 68-year-old male who presented to the emergency department with vague abdominal pain and 30 lb. weight loss. He was found to a pancreatic neck mass by EUS imaging with vascular invasion, eliminating the possibility of complete resection. Rapid onsite evaluation showed malignant cells with granular and clear cell features (Figure 1). Luckily a cell block was obtained. The differential diagnosis included: pancreatic clear cell ductal adenocarcinoma, acinar cell carcinoma, and metastatic renal cell carcinoma. Based upon the clinical, cytological and endoscopic findings, it was initially thought that the lesion in question was an acinar cell carcinoma. Immunohistochemical stains for acinar/neuroendocrine markers were performed using the cell block but were negative. Before signing out the case as clear cell ductal adenocarcinoma of the pancreas, which is a very rare variant, the cytopathologist decided to order an additional small battery of immunohistochemical stains to determine if the lesion could in fact be a metastatic renal cell carcinoma. Surprisingly, the renal cell markers were positive (Figure 2). Upon further questioning, the patient recalled having “kidney surgery” nearly twenty years prior.

The second case was a 58-year-old male who presented to an outside institution with severe abdominal pain and weight loss. Imaging studies at the outside facility showed a mass in the pancreas. He was referred to our institution for EUS-FNA. At EUS, a 6 x 5cm mass was seen in the pancreatic body invading the celiac artery. The mass was hypoechoic with irregular borders. After one pass, a diagnosis of adenocarcinoma was rendered (Figure 3) and a subsequent pass was performed for cell block. In addition to the previously noted adenocarcinoma (Figure 4) the cell block also showed malignant squamous cells. Based on this fortuitous cell block finding, a final diagnosis of adenosquamous carcinoma of the pancreas was made.

These two cases are shining examples of where the cell block did not initially appear to be necessary, but ultimately made a profound impact on the final diagnosis. Some may claim that a diagnosis of “cancer” is sufficient. Unfortunately, the decades-long war on cancer has taught us that not all cancers are created equal. Using the first example from above stage IV clear cell renal cell carcinoma has a poor prognosis with an 11% five-year survival. In contrast the five-year survival for pancreatic adenocarcinoma irrespective of stage is 6%. While both entities carry a grim prognosis metastatic renal cell carcinoma has nearly twice the five year survival of pancreatic adenocarcinoma. Furthermore, treatment modalities and targeted molecular therapies are highly dependent upon a clear delineation of tumor subtype. In the second case the diagnosis of adenosquamous carcinoma portends a slightly different prognosis compared to the more conventional adenocarcinoma of the pancreas. Aside from being rare, adenosquamous carcinoma is a much more aggressive subtype with a median survival of five months. Typically it is highly necrotic and hypervascular and has a greater potential to metastatic spread. In addition, paraneoplastic hypercalcemia can be associated with adenosquamous carcinoma of the pancreas, which may increase the patient’s morbidity. So, while the majority of pancreatic adenocarcinomas can effectively and accurately be diagnosed in the

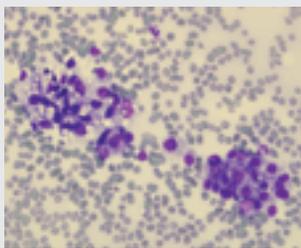


Figure 1: Neoplastic cell groups with granular and vacuolated cytoplasm. The nuclei were slightly irregular and the nucleoli were inconspicuous.

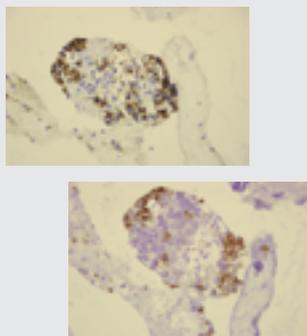


Figure 2: RCC and CD10 stain positive in the malignant cells confirming the diagnosis of renal clear cell carcinoma.

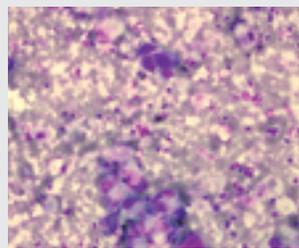


Figure 3: The smears showed poorly preserved cells with mucin in the cytoplasm and some with polygonal cells with well-defined cell borders in a necrotic milieu.

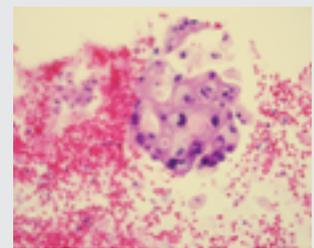


Figure 4: The cell block shows intimately admixed squamous and glandular differentiation with keratinizing cells surrounding glandular cells with signet ring features and mucin droplets.

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absence of a cell block, these two cases illustrate the existence of rare entities that can be mistaken for adenocarcinoma. Fortunately, a good cell block can often sort out the diagnostic “horses from the zebras.”

The Frontier

While treatment options and prognosis have traditionally been dependent upon the anatomic location of the tumor, the clinical stage and the histologic grading, as we enter this molecular age of medicine, therapy is increasingly based on an individual's or a tumor's unique genetic profile. Determining the unique molecular profile utilizing tissue can help to determine which treatment option is optimal for any given patient or tumor. This is particularly important when patients are given adjuvant therapy (chemo/radiation) which may alter the genetic signature of the tumor. A cell block performed at the time of biopsy, prior to adjuvant therapy and resection, can be extremely helpful in determining the original genetic profile of the tumor thus directing specific pharmacogenetic drug therapy.

Immunohistochemical studies performed on cell block tissue could lead to the use of medications designed to target specific molecular pathways. For example, tamoxifen (an estrogen antagonist) is often used to treat estrogen-positive breast carcinoma. Likewise, Herceptin (Trastuzumab) is used in the treatment of breast cancer (and some stomach adenocarcinomas) in which HER2/Neu protein is overexpressed. Gleevec (Imatinib) is a tyrosine kinase inhibitor and has been identified as a multi-therapeutic agent used in the treatment of GIST and chronic myeloid leukemia (CML). Avastin is yet another anti-tumor agent found to be effective in colorectal carcinoma and certain lung, brain and kidney tumors. As we learn more about tumor molecular biology, and identify additional aberrant pathways and mutations, it will be essential to perform molecular analysis on most tumors, thus

making the cell block a vital component in the work up of tumors sampled via fine needle aspiration. Moreover, the material in cell block at our institution is currently used for various research projects which will hopefully lead to new molecular profiles and possible treatment options.

Ending all Road Blocks

The cell block is a highly useful and complementary diagnostic modality. As we have shown, routine use of cell blocks has a variety of benefits including:

- 1. Increased diagnostic sensitivity and specificity**
 - > This is achieved both through morphology as well as through the use of ancillary diagnostic modalities such as immunohistochemistry
- 2. Cell block tissue can be shared for research and/or second opinions without the risk of losing the original diagnostic material**
 - > Since the cell block functions as a traditional paraffin embedded tissue block, multiple slides can be cut from the block while maintaining the integrity of the original diagnostic sample
- 3. Cell block tissue can be used to test for molecular aberrations which may be helpful in guiding existing and future targeted therapies**
 - > As more molecular targets are discovered and corresponding therapies are developed, cell block tissue will allow the oncologist the ability to direct or redirect therapy based on the tumor's characteristic molecular profile

Because the cell block is diagnostically useful, simple to prepare, and cost efficient, there should be no barriers to the routine implementation of this technique during EUS-FNA procedures. It is imperative that the endoscopy team (endoscopist and cytotechnologist/pathologist) recognize the potential impact of collecting such small amounts of tissue on patient diagnosis, treatment and prognosis.

Markers Typically Used in Cell Block to Distinguish Lesions on EUS-FNA

Tumor Site/Type	Common Antigens
Adrenal	CK7, TTF-1 (lung origin), HMB-45 (melanoma), inhibin
Breast	ER, PR, GCFP (gross cystic fluid protein), mammaglobin, CK7, E-cadherin (negative in lobular carcinoma)
Colon	CEA (monoclonal), CK20, CDX-2
GI Tract-Stomach	CEA (monoclonal), CK7 GIST - ckit (CD117), CD34, DOG-1 Schwannoma - S100
Germ Cell	PLAP, CD117 (c-kit), α -fetoprotein, β -HCG, AE1/3, Oct 3/4
Granulomatous dz	AFB, GMS
Hepatic (HCC)	Hepar1, α -fetoprotein, CK7, CD10, pCEA, reticulin
Lung	TTF-1, CK7, CEA, Napsin A, chromogranin, p63
Lymphoma	Leukocyte Common Antigen (CD45) CD3, CD20 Hodgkins - CD15, CD30 Anaplastic Large Cell - CD30, ALK-1 Follicular Lymphoma - CD10
Melanoma	S100, HMB-45, MART1
Ovary	CEA, CA-125
Pancreas	Adenocarcinoma - CA 19-9, CEA Neuroendocrine - Chromogranin, synaptophysin, NSE, CD56, gastrin, somatostatin SPN - α 1-antichymotrypsin, β -catenin, PR, chromogranin (negative) AIP - IgG-4
Renal	EMA, CD10, RCC, vimentin, inhibin (to exclude adrenal cortical)

Additional Reading:

Papers that support cell block utility

BREAST

- **Rapid cell blocks improve accuracy of breast FNAs beyond that provided by conventional cell blocks regardless of immediate adequacy evaluation.**
Akalin A, Lu D, Woda B, Moss L, Fischer A.
Diagn. Cytopathol. 2008;36:523–529.

GRANULOMAS

- **Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis of sarcoidosis.**
Bartheld MB, Veselić-Charvat M, Rabe KF, Annema JT. Endoscopy. 2010 Mar;42(3):213-7.

LUNG

- **Are EUS-FNA and EBUS-TBNA specimens reliable for subtyping non-small cell lung cancer?**
Tournoy KG, Carpriaux M, Deschepper E, van Meerbeeck JP, Praet M. Lung Cancer. 2012 Apr;76(1):46-50.
- **Accuracy of cell typing in nonsmall cell lung cancer by EBUS/EUS-FNA cytological samples.**
Wallace WA, Rassi DM. Eur Respir J. 2011 Oct;38(4):911-7.

LYMPHOMA

- **Diagnosis of deep-seated lymphomas by endoscopic ultrasound-guided fine needle aspiration combined with flow cytometry.**
Stacchini A, Carucci P, Pacchioni D, Accinelli G, Demurtas A, et al.
Cytopathology. 2012 Feb;23(1):50-6.

PANCREAS

- **MUC1 and MUC2 expression in pancreatic ductal carcinoma obtained by fine-needle aspiration.**
Chheng DC, Benson E, Eltoum I, Eloubeidi MA, Jhala N, Jhala D, et al.
Cancer 2003; 99:365-71.
- **Value of EUS-FNA cytological preparations compared with cell block sections in the diagnosis of pancreatic solid tumours.**
Kopelman Y, Marmor S, Ashkenazi I, Fireman Z.
Cytopathology. 2011 Jun;22(3):174-8.

SPINDLE CELL (GI)

- **DOG1 antibody is a highly sensitive and specific marker for gastrointestinal stromal tumors in cytology cell blocks.**
Hwang DG, Qian X, Hornick JL. Am J Clin Pathol. 2011 Mar;135(3):448-53.

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ENDO-96801-AA August 2012

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