



Annie Toja. Nice, France, The Old Port. Acrylic.

*The science of pathologic diagnosis
of metastatic malignancies in the
liver has progressed.*

Pathology of Liver Metastases

Barbara A. Centeno, MD

Background: *The liver is the most frequent site of metastatic disease, and metastatic disease to the liver is far more common than primary liver carcinoma in the United States. Pathologic evaluation of biopsy samples is key to establishing a correct diagnosis for patient management. Morphologic and immunoperoxidase studies, which are the standard for pathologic practice, accurately classify most tumors. Subclassification of carcinoma of unknown primary remains problematic.*

Methods: *The author reviewed the literature for articles pertaining to liver biopsy, diagnosis of specific tumor types, utility of immunohistochemical markers, and microarray and proteomic analysis.*

Results: *Sampling of liver lesions is best accomplished by combining fine-needle aspiration and needle core biopsy. Many malignancies have distinct morphologic and immunohistochemical patterns and can be correctly subclassified. Adenocarcinoma of unknown primary remains enigmatic since current immunohistochemical markers for this differential diagnosis lack specificity. Microarray analysis and proteomic analysis of tumors can provide distinct gene or protein expression profiles, respectively, for tumor classification. These technologies can be used with fine-needle aspiration and needle core biopsy samples.*

Conclusions: *Most metastatic malignancies in the liver may be correctly diagnosed using standard morphology and immunohistochemical techniques. However, subtyping of some carcinomas and identification of site of unknown primary remains problematic. New technologies may help to further refine our diagnostic capabilities.*

From Pathology Services at the H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida.

Submitted June 29, 2005; accepted November 30, 2005.

Address correspondence to Barbara A. Centeno, MD, Pathology Services, 12902 Magnolia Drive, MCC Room 2071 H, Tampa FL 33612. E-mail: centenba@moffitt.usf.edu

No significant relationship exists between the author and the companies/organizations who products or services may be referenced in this article.

Abbreviations used in this paper: FNA = fine-needle aspiration, NCB = needle core biopsy.

Introduction

The liver is one of the most common sites for metastatic disease, accounting for 25% of all metastases to solid organs.¹ In the United States and Europe, secondary liver neoplasms are far more common than primary hepatic neoplasms. In the adult oncology patient, most are metastatic carcinomas, of which adenocarcinomas are the predominant subtype, followed by squamous cell carcinomas and neuroendocrine carcinomas.

Other tumor types that metastasize to the liver include melanomas, lymphomas, and rarely sarcomas.

Radiologically, metastatic disease presents as multiple liver lesions, but a solitary liver lesion in the adult oncology patient in the United States is also most likely to be a metastasis. A tissue diagnosis needs to be established before initiating diagnosis, and this is usually accomplished by image-guided sampling using fine-needle aspiration (FNA) or needle core biopsy (NCB) techniques of the liver lesion. Adequate sampling of the lesion is key to obtaining a diagnosis. Key issues for the pathologist evaluating the biopsy sample are determining the tumor type, distinguishing metastatic carcinoma from primary hepatocellular carcinoma or primary cholangiocarcinoma, and determining the site of primary origin. The pathologist uses morphology to establish a differential diagnosis and then uses semi-quantitative immunohistochemical studies to refine the diagnosis, but these techniques have limitations.

Microarray analysis and proteomic analysis have been used to establish tumor classifiers. Early studies have demonstrated the feasibility of using these on FNA and NCB samples. These technologies will improve our ability to subclassify tumors of unknown primary.

The topic of liver metastases is broad. This discussion focuses on the most significant tumor types in adult oncology patients, with an emphasis on differential diagnosis of carcinoma of unknown primary.

Sampling Technique and Preparation

In order to establish a treatment regimen, tissue diagnosis of liver masses is required. FNA and NCB using either transabdominal ultrasound or computed tomography scanning guidance are the most frequently used modalities and provide diagnostic specimens in over 90% of cases.² Endoscopic ultrasound-guided FNA (EUS-FNA) is being used more frequently to aspirate lesions in the left lobe of the liver. The decision to use FNA or NCB depends on the size and location of the lesion, the suspected diagnosis, and the risk of complications. The experience of a radiologist performing the biopsy is also an important factor. The availability of cytologists to evaluate the FNA for adequacy may also play a role in selection of sampling technique.

A misconception among some clinicians is that NCB is better than FNA because it procures more tissue. However, studies in the literature and personal experience indicate that both are complementary, and an adequate FNA with a well-prepared cellblock can provide sufficient tissue for immunohistochemical studies.

In one recent study of 141 patients with abdominal lesions sampled with both FNA and NCB, FNA proved more sensitive than NCB at diagnosing malignancy (86.1% vs 80.6%, respectively).³ In other studies evalu-

ating FNA and NCB of abdominal organs, similar results have been shown by other authors.^{4,9} All of these studies showed the sensitivity of FNA to be 2% to 24% greater than that of NCB. The combination of FNA and NCB increased the overall sensitivity. A few studies have provided results contradicting these findings.¹⁰⁻¹² However, none of these studies used on-site immediate assessment of FNA samples by a cytologist, which has been shown to maximize diagnostic yield and accuracy.¹³⁻¹⁵

A well-prepared cellblock derived from an FNA sample produces a microhistology specimen that can provide sufficient material to evaluate for architectural features and to perform immunohistochemical studies.^{2,16-18} This provides an alternative to NCB when an NCB cannot conveniently be performed. If only an NCB will be obtained, then cytologic evaluation of touch preparations of the cores can provide similar rapid assessments of specimen adequacy.¹⁹ Touch imprints of core biopsies may be used to minimize the number of biopsy procedures needed²⁰ by ensuring that the NCB contains diagnostic material.

In summary, selection of guidance and biopsy technique depends on the location and site of the lesion, the potential for complications using either technique, and the experience and expertise of the radiologist. FNA and NCB are complementary. On-site cytologic assessment of FNA or NCB using touch imprints improves the adequacy of both.

Establishing the Tumor Type

Correct clinical history is crucial to the pathologic interpretation of a biopsy from a liver mass suspected of being a metastasis. The most accurate interpretation is rendered when the history of previous cancer and other pertinent findings, such as radiological findings and serum tumor marker levels, are provided for correlation with the pathologic findings.

The first step when evaluating a liver biopsy is to establish the general tumor type, ie, whether the neoplasm is a carcinoma, sarcoma, lymphoma, or melanoma. Morphology alone is often diagnostic, but frequently ancillary studies are needed for definitive diagnosis when the tumor is poorly differentiated. A panel incorporating at least cytokeratins, S100, and leukocyte-common antigen (LCA) will assist in subcategorizing the neoplasms. Carcinomas express cytokeratins, most lymphomas (except anaplastic large-cell lymphoma) express LCA, and S100 is the most sensitive marker for the diagnosis of melanoma. Additional antibodies can be added once the differential diagnosis has been narrowed down.

Carcinomas

Carcinomas are the most frequent source of metastases to the liver. Lung, colon, pancreas, breast, and stomach

are the most frequent sources, accounting for 24.8%, 15.7%, 10.9 %, 10.1% and 6.1%, respectively, of all patients with metastatic disease in one autopsy series.²¹ Ovarian, endometrial, prostate, and urothelial carcinomas are less frequent sources of metastases, each accounting for 4% or less.²¹

The appearance of carcinomas depends on their differentiation and includes adenocarcinomas, squamous cell carcinoma, urothelial carcinoma, neuroendocrine carcinomas, mixed types of carcinomas such as adenosquamous carcinoma, or specific types such as adrenal cortical carcinoma, renal cell carcinoma, and hepatocellular carcinoma.

Squamous Cell Carcinoma

Squamous cell carcinoma is an uncommon metastasis to the liver. Possible primary sites include lungs, esophagus, head and neck, genital primaries, or anorectal primaries. FNA smears show polygonal cells occurring singly and in groups with hyperchromatic, irregular

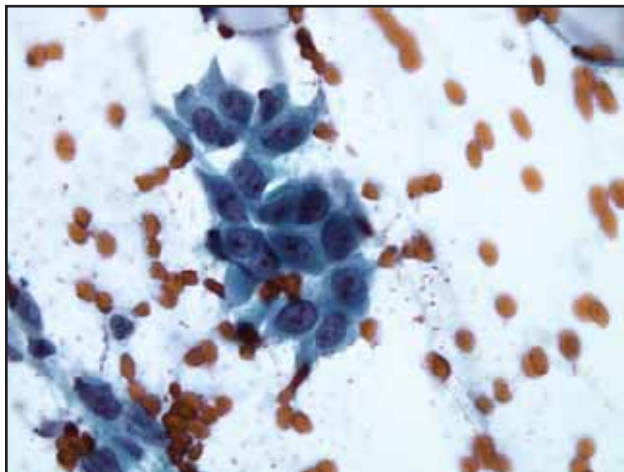


Fig 1. — Squamous cell carcinoma, FNA. This sheet of cells has dense polygonal cytoplasm with junctions. The nuclei show an increased nuclear to cytoplasmic ratio and atypia (Papanicolaou, × 63).

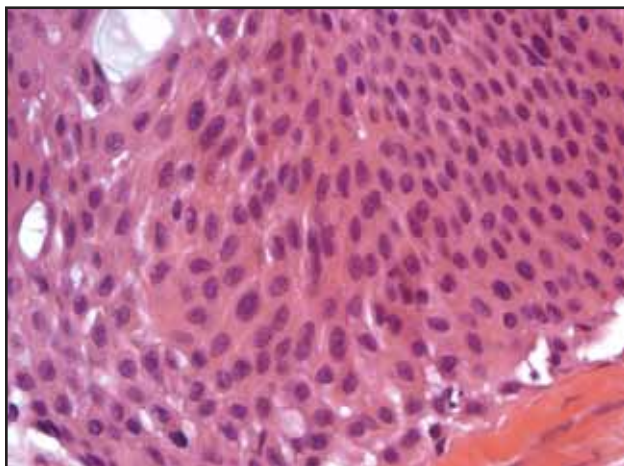


Fig 2. — Squamous cell carcinoma, core biopsy. The cells have abundant, dense, polygonal eosinophilic cytoplasm. Intercellular junctions between the cells are evident (hematoxylin-eosin, × 40).

nuclei. The cytoplasm is dense and nonvacuolated, in contrast to that of adenocarcinoma (Fig 1). Keratinized cells will have orangeophilic cytoplasm on Papanicolaou-stained smears. Histopathology specimens will show cells with dense, polygonal, eosinophilic cytoplasm with intercellular desmoplastic junctions (Fig 2). The presence of keratinization confirms the diagnosis, but it is not always evident. The morphology of squamous cell carcinoma is not specific to site of origin. History is key in identifying the primary site since immunohistochemical studies are not helpful.

Urothelial Carcinoma

The cells on cytology smears are arranged in discrete and small syncytial cell clusters. The nuclei are central to eccentric, and the cytoplasm is variable. A key feature is the presence of cercariform cells (Fig 3). These are cells with nucleated globular bodies and unipolar nontapering cytoplasmic process.²² The pattern on histomorphology is varied. The cells are typically arranged in sheets and have dense, amphophilic cytoplasm. The nuclei are typically elongated and may show grooves (Fig 4).

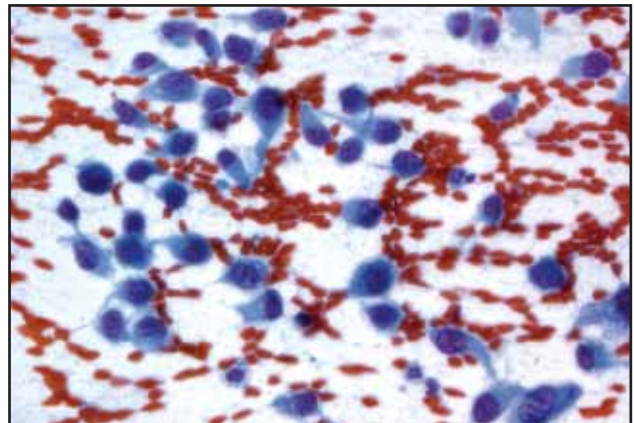


Fig 3. — Urothelial carcinoma, FNA. The cells are dispersed as single cells. Many of the cells have a bulbous head and a tail, characteristic of cercariform cells (Papanicolaou, × 60).

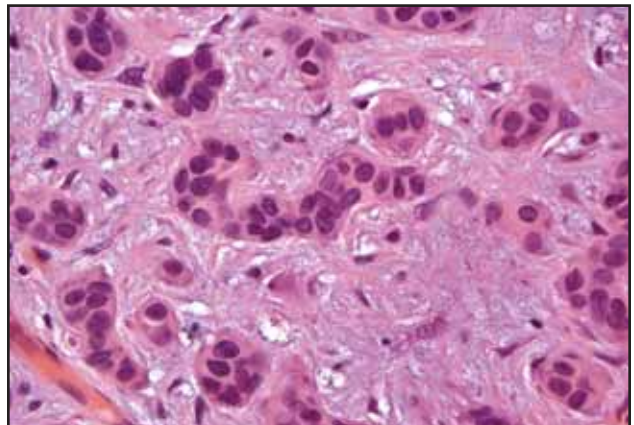


Fig 4. — Urothelial carcinoma, core biopsy. The cells are arranged in cohesive nests and have abundant eosinophilic cytoplasm. The nuclei show nuclear grooves, a feature of urothelial carcinoma (hematoxylin-eosin, × 40).

Neuroendocrine Carcinomas

Neuroendocrine carcinomas vary in the degree of differentiation. Low-grade tumors such as carcinoids have a monomorphic appearance with minimal mitotic activity and no necrosis. Smears are uniformly cellular and composed of a monomorphic population of tumor cells. A tumor with a plasmacytoid appearance on cytology samples is virtually pathognomonic (Fig 5). Histopathology samples will show similar features. The chromatin shows a characteristic salt and pepper appearance on both smears and core biopsy samples (Fig 6). Carcinoids may arise anywhere in the gastrointestinal tract. Pancreatic endocrine tumors have identical morphologic features. High-grade tumors, such as metastatic small-cell carcinoma from the lung, will show nuclear molding, necrosis, and abundant mitotic activity. The chromatin is diffusely and finely stippled (Figs 7 and 8).

Immunohistochemical studies are useful for the identification of a neoplasm as showing neuroendocrine differentiation. The standard panel is synap-

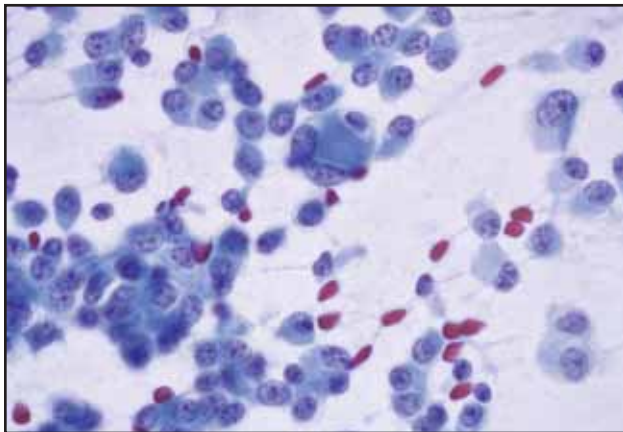


Fig 5. — Carcinoid tumor, FNA. The cells have abundant amphophilic cytoplasm and the nuclei are eccentrically placed, imparting a plasmacytoid appearance. Some cells are binucleated. The cells exhibit the characteristic salt and pepper chromatin pattern (Papanicolaou $\times 40$).

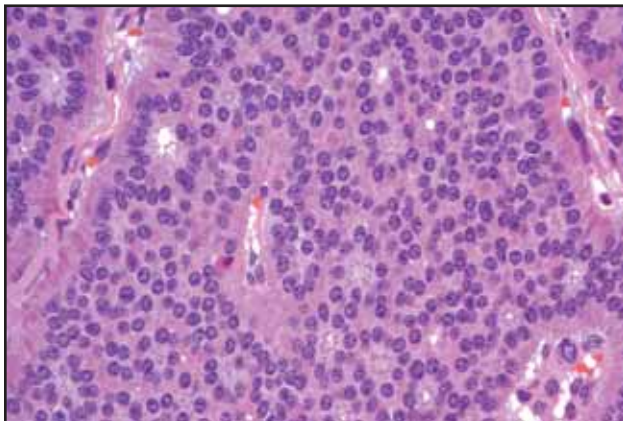


Fig 6. — Carcinoid tumor, core biopsy. The cells are arranged in solid nests with focal areas of palisading or acinar structure formation. The nuclei are round and uniform with a salt and pepper chromatin (hematoxylin-eosin, $\times 40$).

physin, chromogranin, and neural cell adhesion molecules (NCAM [CD56]). Immunohistochemical studies are less helpful for the identification of neuroendocrine carcinoma of unknown primary.²³

Adenocarcinomas

Adenocarcinomas are the most significant since they are the most frequent type of carcinoma to metastasize to the liver. Lung, colon, pancreas, breast, and stomach are the most frequent, representing 24.8%, 15.7%, 10.9%, 10.%, and 6.1% of cases, respectively, in one autopsy series.²¹ Ovary, endometrial, prostate, cholangiocarcinoma, and thyroid are less frequent, each accounting for less than 4%.²¹ Adenocarcinomas are also the most frequent type of carcinoma presenting as unknown primary in the liver in the adult oncology patient.^{24,25}

Adenocarcinomas are neoplasms derived from glandular tissues. The most frequent appearance of adenocarcinomas is columnar cells forming acinar structures, which recapitulate the gland formation within

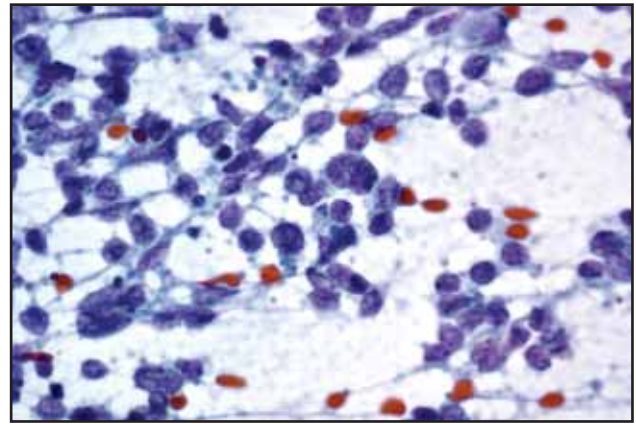


Fig 7. — Small cell carcinoma, FNA. In contrast to the carcinoid tumor, these cells exhibit greater variation in nuclear contour. The cells have scant amphophilic cytoplasm. Nuclear molding, crush artifact and necrosis are evident. The salt and pepper chromatin pattern typical of neuroendocrine neoplasms is retained (Papanicolaou, $\times 63$).

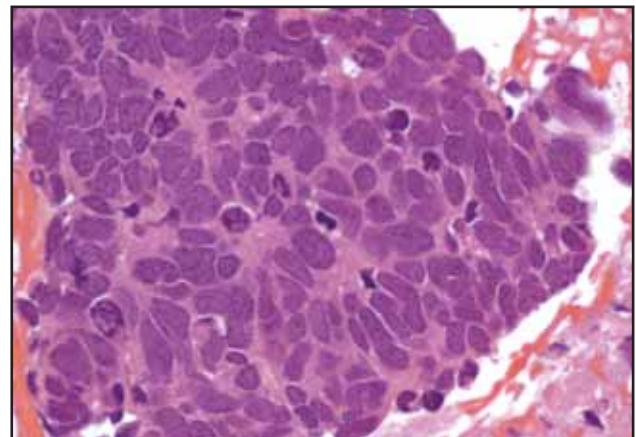


Fig 8. — Small cell carcinoma, FNA, cell block. The cells have scant, eosinophilic cytoplasm. The nuclei are elongated and fusiform. Apoptosis is noted (hematoxylin-eosin, $\times 40$).

the normal organ (Fig 9). The typical adenocarcinoma shows focal mucin production, within either the cytoplasm or lumen, which can be demonstrated with a histochemical stain for mucin, such as mucicarmine.

Morphologic subtypes include the mucinous carcinoma or colloid-type carcinoma and signet ring cell carcinoma. While the morphologic pattern of most adenocarcinomas is not specific for site of origin, some primary sites have characteristic features that lead to their recognition on FNA or NCB specimens. These include colorectal carcinoma, breast carcinoma, and pancreatobiliary carcinoma.

A key feature of colorectal carcinoma is a dirty, necrotic background on cytology smears (Fig 10). The cells are columnar in appearance. Histopathology will show an adenocarcinoma with abundant central necrosis in the glands (Fig 11). Low-grade ductal adenocarcinomas appear as a monomorphic population on cytology smears. The groups are flat and angulated. The monomorphic appearance is evident on histopathology specimens (Fig 12). Lobular carcinoma

forms a dyshesive cell population composed of small cells with eccentric nuclei. Histology specimens will show cells infiltrating in single file pattern. A characteristic feature of both types of mammary carcinomas, associated most often with lobular carcinoma, is cells with a targetoid cytoplasmic lumen (Fig 13).

Pancreatobiliary carcinomas do not exhibit a specific pattern on cytology smears, although an adenocarcinoma with abundant cytoplasmic mucin or clear nuclei may be suggestive. They are typically associated with abundant sclerotic stroma (Fig 14), and therefore this possibility may be suggested on histopathology specimens. However, primary intrahepatic cholangiocarcinoma is also associated with sclerotic stroma, so the distinction of primary cholangiocarcinoma from metastatic pancreatobiliary carcinoma cannot be made without the history.

The morphologic patterns of other types of adenocarcinomas such as those originating in the lungs, endometrium, esophagus, or intestinal type of gastric carcinoma do not have any specific features.

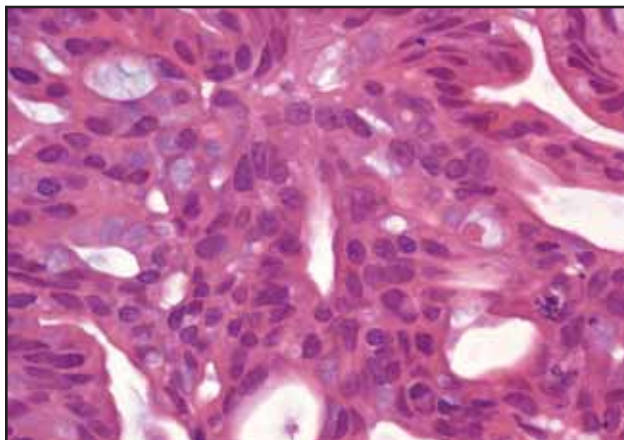


Fig 9. — Adenocarcinoma, NOS, core biopsy. The cells are arranged in an acinar pattern, characteristic of adenocarcinoma from any site. Pale, bluish mucin is evident the cytoplasm of some cells and lumen (hematoxylin-eosin, $\times 40$).

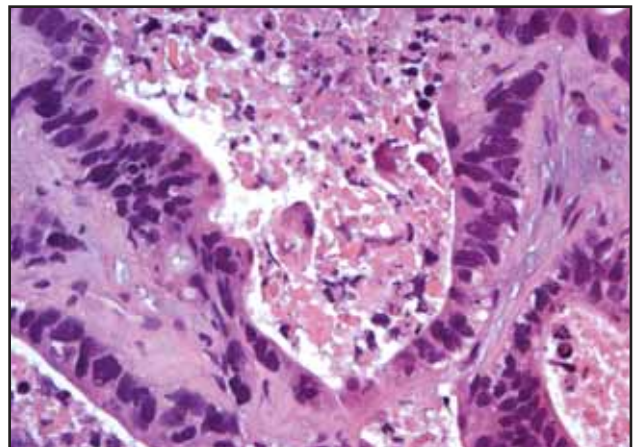


Fig 11. — Colonic adenocarcinoma, core biopsy. The glands show abundant central necrosis, a characteristic feature of colonic adenocarcinoma (hematoxylin-eosin, $\times 40$).

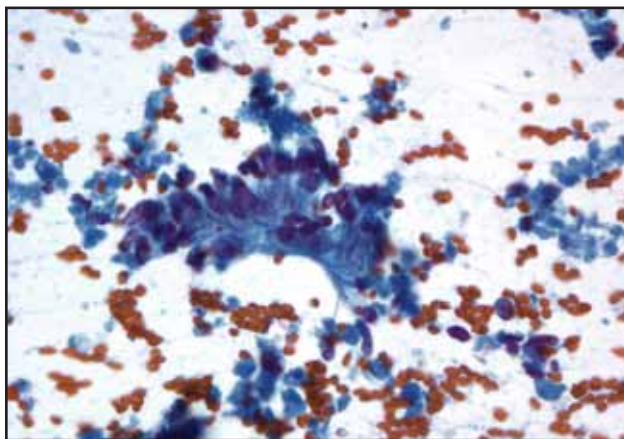


Fig 10. — Colonic adenocarcinoma, FNA. The smear shows columnar cells arranged in a palisaded pattern. The background shows abundant necrosis (Papanicolaou, $\times 40$).

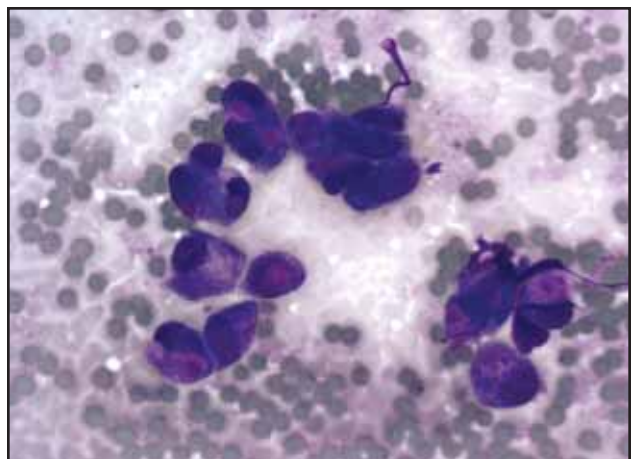


Fig 12. — Mammary carcinoma, FNA. The cells are dispersed and show eccentric nuclei. A number of cells have intracytoplasmic mucin vacuoles (Diff-Quik, $\times 60$).

Mucinous Carcinoma

Mucinous carcinoma is a morphologic subtype of adenocarcinoma, defined as a carcinoma that contains more than 50% extracellular mucin. This subtype is not specific to any organ. Mucinous carcinomas are most frequently identified in the colon, but they also occur in

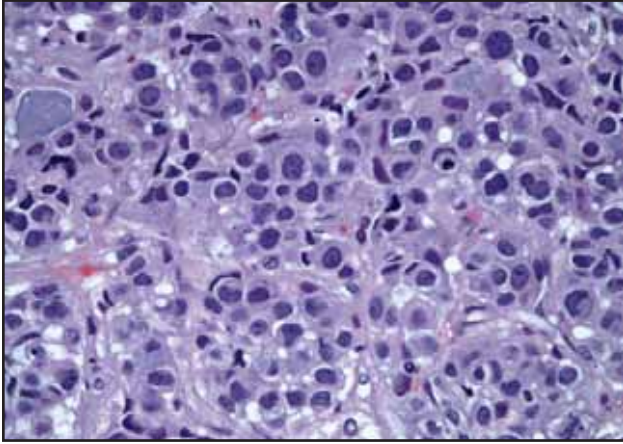


Fig 13. — Mammary carcinoma, core biopsy. The cells are arranged in rounded nests (hematoxylin-eosin, $\times 40$).

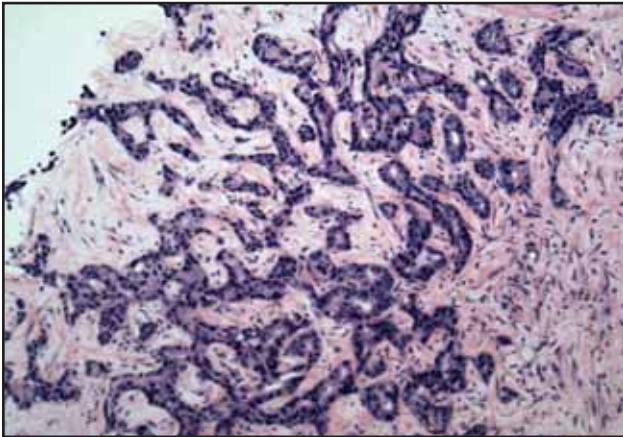


Fig 14. — Metastatic pancreatic carcinoma. The carcinoma is surrounded by a desmoplastic stroma (hematoxylin-eosin, $\times 20$).

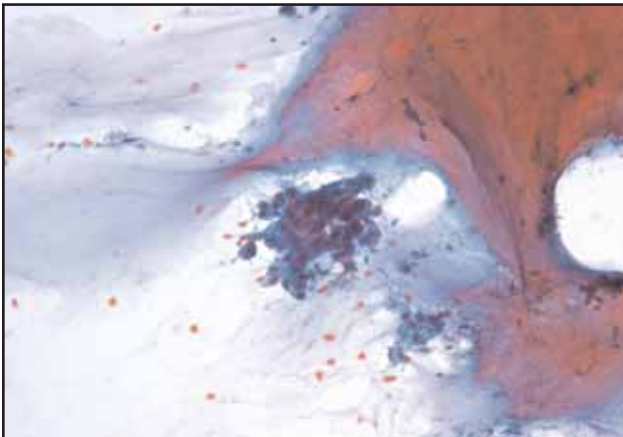


Fig 15. — Mucinous carcinoma, FNA. The cluster of malignant cells floats in a background of viscous mucin (Papanicolaou, $\times 40$).

the breast, ovaries, and pancreas and may arise anywhere in the gastrointestinal tract. Mucinous bronchoalveolar carcinoma has similar features. A mucinous carcinoma in the liver is most likely to be of colorectal origin, but other primary sites need to be considered. Aspirates show malignant glandular cells floating in pools of mucin (Fig 15). The two histologic patterns are tumor cells floating in pools of mucin or pools of mucin partially lined by tumor cells (Fig 16). The tumor cells are mucin-producing columnar cells or cells that contain a single large vacuole.

Signet Ring Cell Carcinoma

Signet ring cell carcinoma shows single cells with a cytoplasmic mucin vacuole that displaces the nucleus. The nucleus is sharply angulated at the tips (Fig 17). Gastric carcinoma is most commonly associated with this morphology, but as for mucinous carcinomas, it may arise in any organ in the gastrointestinal tract. The differential diagnosis includes metastatic lobular breast carcinoma. An immunohistochemical panel that

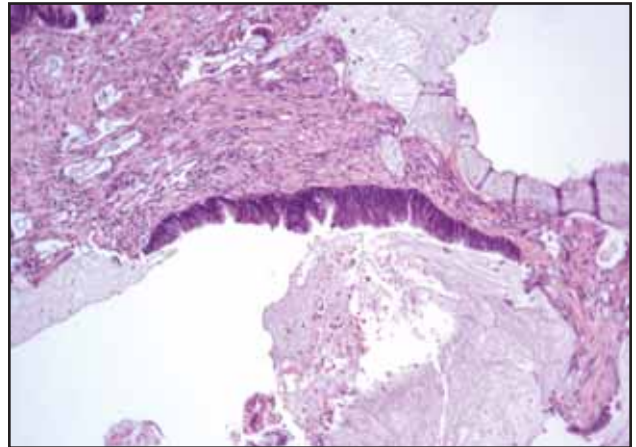


Fig 16. — Mucinous carcinoma, biopsy. The carcinoma is composed predominantly of extra cellular mucin. The malignant glands cling to the stroma and surround pools of mucin (hematoxylin-eosin, $\times 20$).

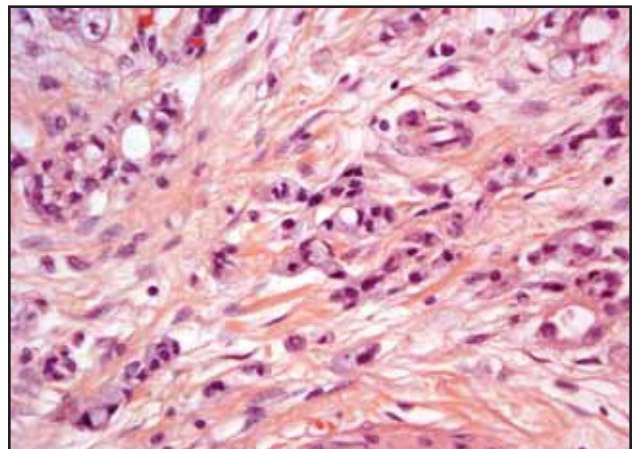


Fig 17. — Signet ring cell carcinoma, core biopsy. Signet ring cells with eccentric, elongated nuclei displaced by intracytoplasmic mucin vacuoles (hematoxylin-eosin, $\times 40$).

includes CK7, CK20, estrogen receptors,²⁶ and GCDFP15 (BRST2)^{27,28} can help with this differential diagnosis. Lobular carcinoma will express CK7, ER, and GCDFP15 and usually does not express CK20. Progesterone receptor is not as specific as estrogen receptor since its expression was identified in other carcinomas, including gastric/esophageal.²⁹

Adenosquamous Carcinoma

Adenosquamous carcinoma is composed of a mixture of squamous carcinoma and adenocarcinoma, as the name implies. One of the components must comprise at least 30% of the tumor. This type of carcinoma may arise anywhere in the gastrointestinal tract, including the pancreas and biliary system, and also the lungs. Since primary cholangiocarcinoma may show a similar morphology, it will not be possible to determine whether it is primary or metastatic without clinical history.

Renal Cell Carcinoma

Renal cell carcinoma infrequently metastasizes to the liver, but it accounts for 3% of all metastases in one

autopsy series.²¹ The classic appearance is that of a carcinoma composed of clear cells arranged in nests with intervening stroma and blood vessels (Fig 18). Variants include papillary renal cell carcinoma and chromophobe renal cell carcinoma. The cytoplasm of FNA will show polygonal cells arranged singly and in clusters. The nuclei are round with prominent nucleoli, and the cytoplasm is clear or granular. Large groups or sheets of cells are arranged along transgressing endothelium (Fig 19), a pattern that mimics hepatocellular carcinoma on aspirates.³⁰ Papillary renal cell carcinoma will not demonstrate prominent nucleoli or clear cytoplasm. The cytoplasm in chromophobe renal cell carcinoma is balloon-like and excessive. The pattern of clear cell renal cell carcinoma may mimic that of hepatocellular carcinoma, particularly the clear cell variant of hepatocellular carcinoma.

Adrenal Cortical Carcinoma

Adrenal cortical carcinoma is a rare neoplasm. The liver is one of its most common sites of metastasis.³¹ It merits mention because its morphologic pattern overlaps

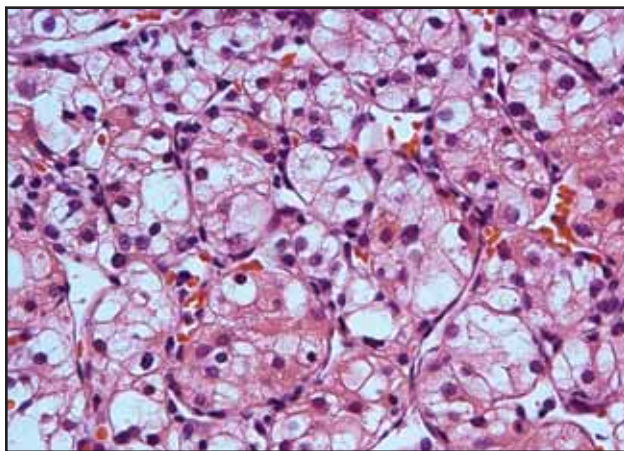


Fig 18. — Renal cell carcinoma, biopsy. Clear cells surround by vascular stroma (hematoxylin-eosin, $\times 40$).

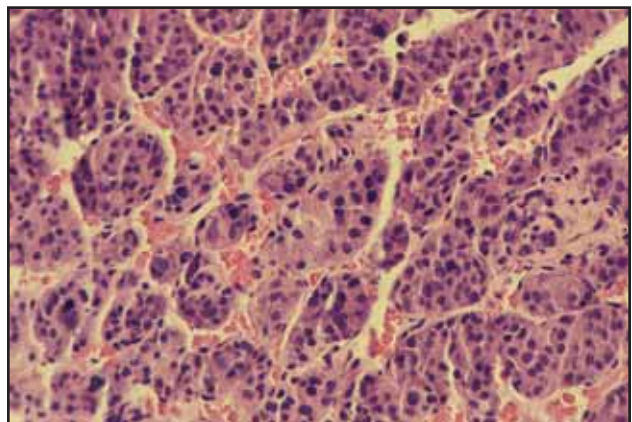


Fig 20. — Adrenal cortical carcinoma, biopsy. The cells clusters show endothelial wrapping, similar to that shown by hepatocellular carcinoma (hematoxylin-eosin, $\times 20$).

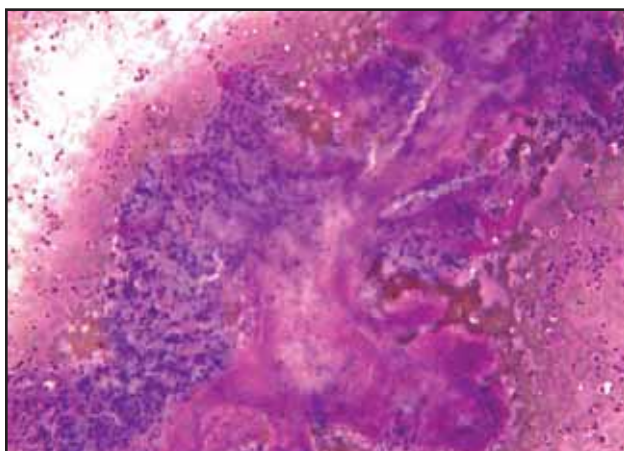


Fig 19. — Renal cell carcinoma, FNA. This fragment has a central, transgressing vasculature, which mimics the pattern seen in hepatocellular carcinoma. Stripped nuclei are seen in the background (Diff-Quik, $\times 20$).

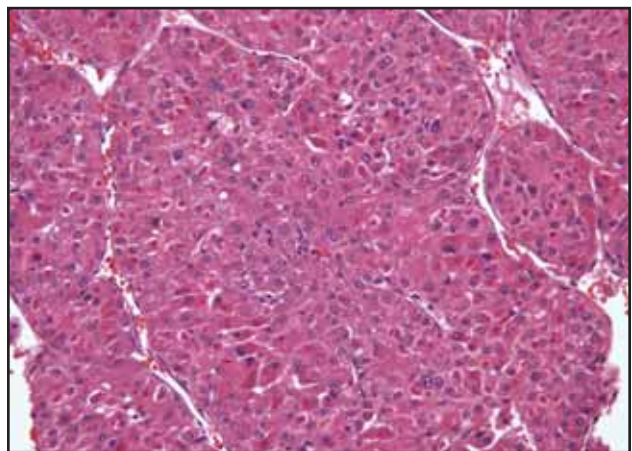


Fig 21. — Hepatocellular carcinoma, biopsy. Classic pattern of hepatocellular carcinoma showing widened trabeculae and intracytoplasmic inclusions (hematoxylin-eosin, $\times 20$).

with that of hepatocellular carcinoma on FNA and NCB.³² The cells of adrenal cortical carcinoma are polygonal in shape, like those of hepatocellular carcinoma. Furthermore, on core biopsy samples, it may show endothelial wrapping, similar to that produced by hepatocellular carcinoma³⁰ (Figs 20 and 21). Smears show polygonal cells arranged singly and in clusters without transgressing endothelium. The nuclei are hyperchromatic and variable in size. Prominent nucleoli are not a feature as they are for hepatocellular carcinoma.

Melanomas

Melanomas are known as the great mimickers in pathology. Generally, melanoma must be included in the differential diagnosis of almost any neoplasm since its appearance can be so varied and also since patients may present with liver metastases many years after the diagnosis of the primary tumor. However, it is a relatively infrequent source of metastases in the liver, accounting for approximately 2.2%.²¹ A single cell population with cytoplasmic melanin pigment is diagnostic on FNA smears (Fig 22), but nonpigmented or amelanotic melanomas are difficult to diagnose. Biopsies typi-

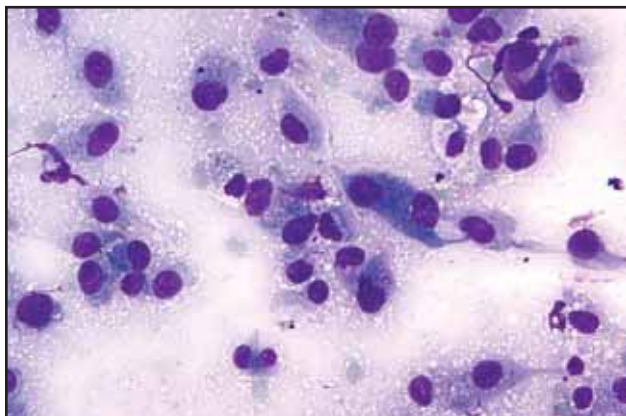


Fig 22. — Melanoma, FNA. The smear shows a dispersed cell population. The nuclei vary in size and shape. Some of the cells show black melanin pigment in the cytoplasm (Diff-Quik, $\times 40$).

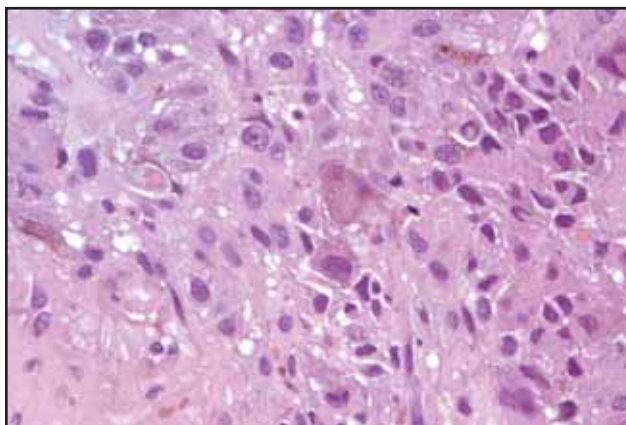


Fig 23. — Melanoma, core biopsy. The malignant cells have atypical nuclei with prominent nucleoli, a feature considered typical of melanoma. Melanin pigment is visible (hematoxylin-eosin, $\times 40$).

cally show a population of pleomorphic cells with intranuclear inclusions and prominent nucleoli (Fig 23). Immunohistochemistry is specific and sensitive for the diagnosis of melanoma. Melanomas are S100-positive, HMB45 and MelanA-positive. A new antigen cocktail consisting of HMB45, tyrosinase, and MART-1 is sensitive for the diagnosis of melanoma.³³ However, S100 remains the most sensitive for detection of melanoma, although it lacks specificity.³⁴

Lymphomas

Lymphomas are dyshesive neoplasms on FNA smears. The background of the smears shows lymphoglandular bodies (Fig 24) indicating that the neoplasm is of lymphoid origin, but they are not specific for benignancy or malignancy. The appearance depends on the specific type. Large-cell lymphomas, the most frequent type of lymphoma to secondarily involve the liver, are usually easy to recognize as malignant because they are composed of large lymphocytes (greater than 3 times the size of a normal lymphocyte) with nuclear membrane irregularities (Fig 25). Follicular lymphomas, mucosa-

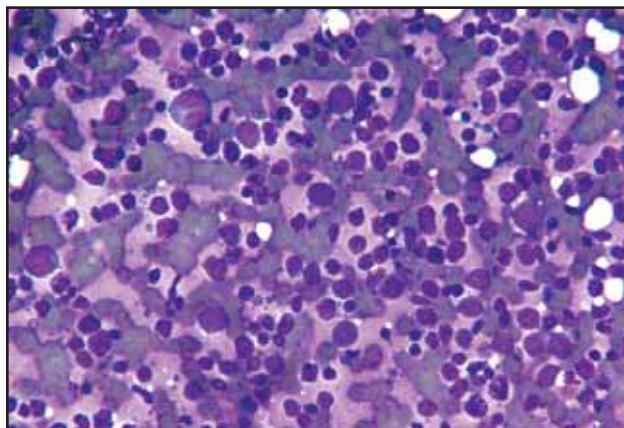


Fig 24. — Large cell lymphoma, FNA. The malignant cells are dispersed. The nuclei are three times the size of normal lymphocytes. The background shows lymphoglandular bodies, which are characteristic for lymphoid processes (Diff-Quik, $\times 63$).

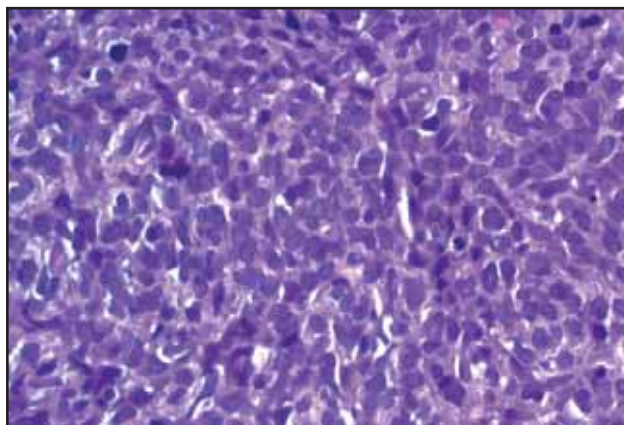


Fig 25. — Large cell lymphoma, biopsy. The specimen shows a population of cells with scant cytoplasm, irregular nuclear membranes and prominent nucleoli (hematoxylin-eosin, $\times 40$).

associated lymphoma tissue (MALT) lymphomas, and small lymphocytic lymphomas are difficult to recognize on morphology alone but can be suspected if there is a previous history of lymphoma. Flow cytometry and gene rearrangement studies can be performed on samples obtained from FNA. Typically, if lymphoma is suspected, an aspirate directed for flow cytometry is requested. NCB can be used for histomorphological grading of follicular lymphomas and immunohistochemical studies. The accuracy of subclassifying lymphomas using cytomorphological and flow cytometric immunophenotyping has been demonstrated.³⁵⁻³⁷

Sarcomas

Sarcomas are usually characterized by a spindle cell appearance. Gastrointestinal stromal tumors and leiomyosarcomas are the most frequent sarcomas to metastasize to the liver.

Aspirates of gastrointestinal stromal tumors demonstrate relatively monomorphic and uniform spindle cells in loose aggregates and singly. The cells may be associated with a myxoid stroma (Fig 26). Occasionally they show epithelioid features, in which case the differential diagnosis includes melanoma, carcinomas,

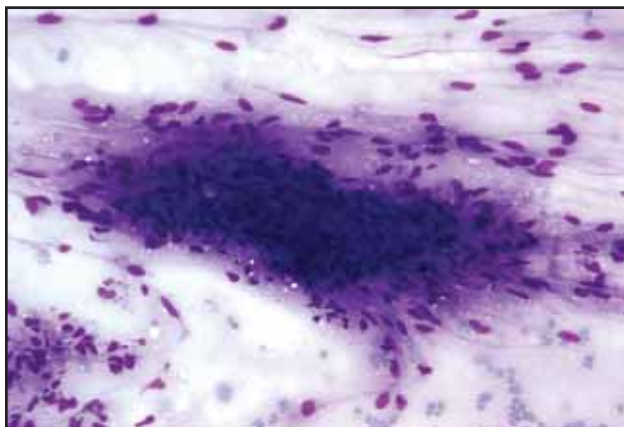


Fig 26. — Gastrointestinal stromal tumor, FNA. This field demonstrates spindle cells embedded in myxoid stroma (Diff-Quik, × 20).

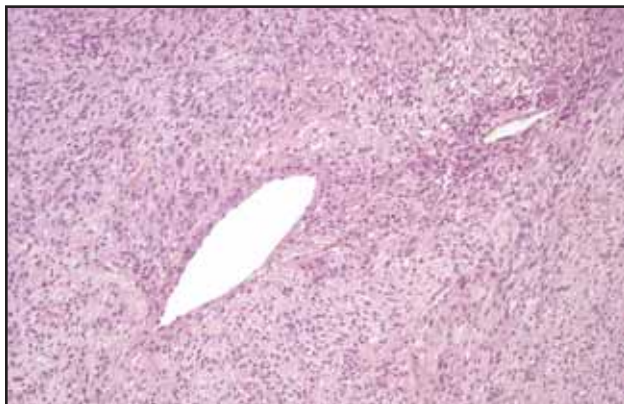


Fig 27. — Gastrointestinal stromal tumor, core biopsy. The plump spindle cells tumor vascularity is prominent. Skenoid fibers are noticeable at this power (hematoxylin-eosin, × 20).

and neuroendocrine tumors. Histopathology samples demonstrate a spindle cell neoplasm with a storiform pattern, prominent vascularity, and occasionally skenoid fibers (Fig 27). The tumors may demonstrate variable amounts of myxoid stroma. Paranuclear vacuoles are a frequent feature (Fig 28). Immunohistochemical analysis shows tumor cell expression for C-kit, CD34, and vimentin. The tumors are typically negative for actin or desmin.³⁸ C-kit expression is diagnostic for these tumors.

Leiomyosarcoma is the most common sarcoma to metastasize to the liver. It shows greater pleomorphism and less vascularity compared to gastrointestinal stromal tumor (GIST) (Fig 29). The characteristic immunophenotype is desmin and smooth muscle actin expression and no C-kit expression.³⁸

Diagnostic Dilemmas

Problems facing the pathologist when evaluating a biopsy include distinguishing hepatocellular carcinoma from other carcinomas with similar features (renal cell

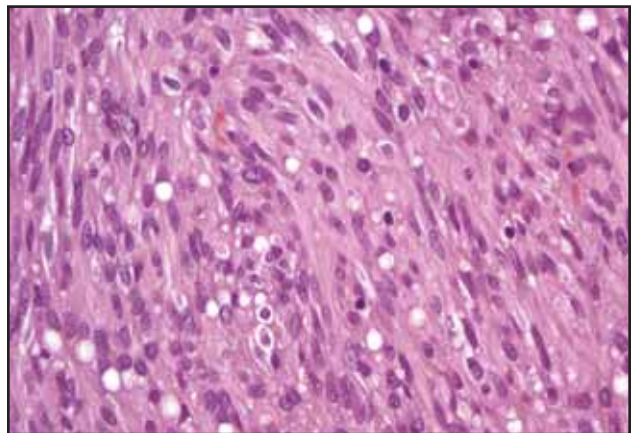


Fig 28. — Gastrointestinal stromal tumor, core biopsy. The cells are spindle in shape with minimal nuclear atypia. Perinuclear halos are evident (hematoxylin-eosin, × 40).

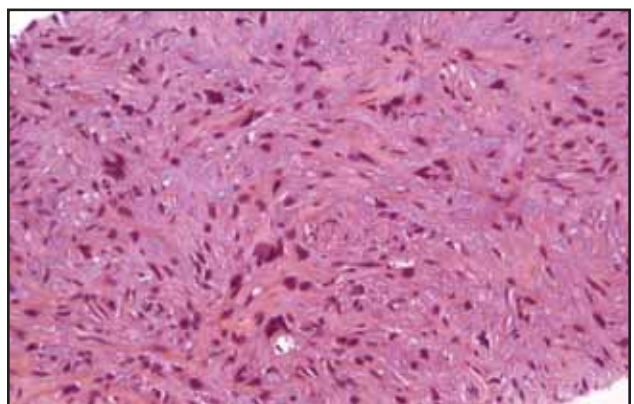


Fig 29. — Leiomyosarcoma, core biopsy. The cells show a greater degree of nuclear pleomorphism and are surrounded by a myxoid stroma. The tumor is less vascular than the GIST (hematoxylin-eosin, × 40).

Table 1. — Immunohistochemical Panel for the Differential Diagnosis of Hepatocellular Carcinoma, Renal Cell Carcinoma, Adrenal Cortical Carcinoma, and Melanoma

Antigen	Tumor Type			
	Hepatocellular Carcinoma	Renal Cell Carcinoma	Adrenal Cortical Carcinoma	Melanoma
HMW CK	—	+	—	—
LMW CK	+	+	—/few cells +	—
Vimentin	+/-	+	+	+
EMA	+	+	—	—
CD 10	Canalicular	+	—	—
AFP	+	—	—	—
HepPar	+	—	—	—
MART-1	—	—	+	+
S100	—	—	—	+
HMB45	—	—	—	+
Synaptophysin	—	—	+	—
Calretinin	—	—	+	—

HMW CK = high-molecular-weight cytokeratin
LMW CK = low-molecular-weight cytokeratin

and adrenal cortical carcinoma) and distinguishing hepatocellular carcinoma from adenocarcinoma. A special problem in the oncology patient is the workup of carcinoma of unknown primary.

Primary Hepatocellular Carcinoma vs Metastases

The morphologic features of renal cell carcinoma and adrenal cortical carcinoma overlap with those of hepatocellular carcinoma, on both cytology smears and histopathology samples. Immunohistochemical analysis differentiates among these three carcinomas. Adrenal cortical carcinomas are weakly positive for cytokeratin and express vimentin and synaptophysin. MART-1, also known as melanA, and usually expressed by melanoma, is useful for diagnosing adrenal cortical carcinoma.^{39,40} Adrenal cortical carcinoma also expresses inhibin, which is slightly more sensitive but less specific.⁴¹ Calretinin has also been shown to be expressed by adrenal cortical cells and neoplasms.⁴²⁻⁴⁴ Clear cell carcinoma of the kidney, the most typical type of renal neoplasm, expresses cytokeratin, vimentin, epithelial membrane antigen (EMA), renal cell carcinoma antibody, and CD10.⁴⁵⁻⁴⁷ Exceptions are the papillary variants and chromophobe cell variants, which do not express vimentin. Alpha-methyl CoA racemase (AMACR) is usually expressed by papillary renal cell carcinomas,⁴⁸ and chromophobe renal cell carcinomas demonstrate colloidal iron not demonstrated by hepatocellular carcinoma or adrenal cortical carcinoma. Hepatocellular carcinoma can usually be identified by expression of low-molecular-weight cytokeratin, HepPar, and a canalicular pattern rather than a cytoplasmic pattern of carcinoembryonic antigen (CEA). The canalicular pattern occurs because the CEA will stain the bile duct canaliculi in hepatocellular carcinoma but not the cytoplasm. CD10 will stain hepatocellular carcinoma in a canalicular pattern but not in the cytoplasm, as it does for renal cell carcinoma.⁴⁹

Other unusual metastases that may mimic hepatocellular carcinoma include hepatoid yolk sac tumor, and oxyphilic follicular carcinoma of the thyroid. Hepatoid yolk sac tumors are rare, and follicular carcinoma of the thyroid rarely gives rise to liver metastases. The immunohistochemical approach to this differential diagnosis is summarized in Table 1.

Adenocarcinoma vs Hepatocellular Carcinoma

Typically, the distinction of adenocarcinoma from hepatocellular carcinoma is clear-cut on morphologic grounds. Adenocarcinoma is characterized by the formation of gland-like or tubular structures. The lumens or the individual cells contain mucin. Hepatocellular carcinoma occasionally forms acinar structures resembling adenocarcinoma (Fig 30) or is poorly differentiated, in which case it cannot be distinguished from adenocarcinoma. In these situations, adjunctive studies are needed. The presence of bile pigment is pathognomonic for hepatocellular differentiation; therefore, if

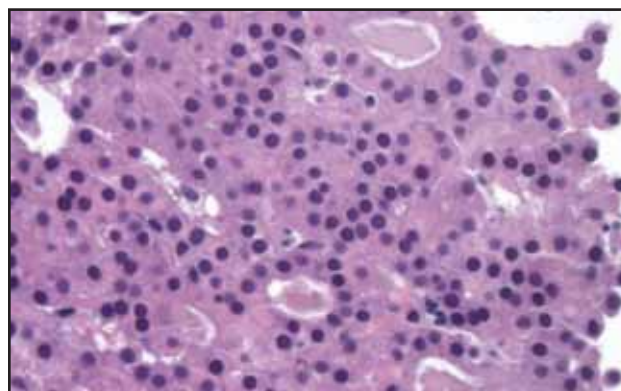


Fig 30. — Hepatocellular carcinoma, biopsy. Acinar pattern demonstrated in hepatocellular carcinoma (hematoxylin-eosin, × 40).

Table 2. — Differential Diagnosis of Hepatocellular Carcinoma From Adenocarcinoma

Antigen	Tumor Type	
	Hepatocellular Carcinoma	Adenocarcinoma
LMW CK	+	+
HMW CK	–/rarely +	+
CEA	Canalicular	+
HepPar	+	–
B72.3	–	+
AFP	+	–
MOC31	–	+
CK19	–	+
Mucicarmine	–	+
Bile	+	–

HMW CK = high-molecular-weight cytokeratin
LMW CK = low-molecular-weight cytokeratin

the pathologist recognizes bile, the diagnosis of hepatocellular carcinoma can be made with certainty. A Hall's stain for bile can help to recognize the bile pigment. Adenocarcinoma typically secretes mucin, so the identification of mucin secretion by the cells using a histochemical stain for mucin, such as mucicarmine, can establish the carcinoma as an adenocarcinoma.

Immunohistochemistry is helpful when morphology and identification of secretory substances fail. Table 2 lists an immunohistochemical panel to facilitate this distinction. Adenocarcinomas express both high- and low-molecular-weight cytokeratin, whereas the cytokeratin expression of hepatocellular carcinoma is usually limited to low-molecular-weight cytokeratin.⁵⁰ More specifically, hepatocytes and hepatocellular carcinoma do not express cytokeratins 1, 5, 10, 11, and 19. Immunohistochemical evaluation for CK19 is particularly useful since adenocarcinomas but not hepatocellular carcinomas express this antigen.⁵¹ As described in the previous section, hepatocellular carcinoma has a specific expression pattern for CEA and CD10 in which they are expressed only in the bile canaliculi.⁴⁹ Adenocarcinomas show a cytoplasmic expression pattern for CEA. MOC31 is reported to be sensitive for the diagnosis of adenocarcinoma.^{52,53} The HepPar antigen is also helpful, but it is expressed occasionally by other tumor types such as adrenal cortical carcinoma, yolk sac tumor, ovarian carcinoma, colonic carcinoma, lung carcinoma, and endocervical carcinoma.⁵⁴ AFP, when expressed, is also specific for hepatocellular carcinoma but is infrequently expressed.⁵⁵ Generally, a panel combining a number of these antigens is most useful for this differential diagnosis.⁵⁵⁻⁵⁷

Primary Site of Origin of Carcinoma

This problem is probably of the greatest significance to the oncologist treating the adult cancer patient and is frequently encountered at our institute. While most patients presenting with metastatic adenocarcinoma have a history of a primary elsewhere, some patients do

not have a known primary. In these cases, the onus falls on the pathologist to navigate the treating physicians to the most likely primary site.

As previously stated, adenocarcinomas are the most frequent type of carcinoma presenting as unknown primary, accounting for approximately 80%.²⁴ Squamous cell carcinomas account for another 15%, and metastases of other tumor types account for the remaining 5%.²⁴ The immunohistochemical workup essentially focuses on adenocarcinomas since the immunohistochemical panels are most applicable to this subtype of carcinoma.

Morphologic clues on the FNA or NCB may help to distinguish some adenocarcinomas from others, as already presented. In a patient with a solitary liver mass, the key will be distinguishing primary cholangiocarcinoma from metastatic adenocarcinoma. Morphology may provide some clues since the association with a densely sclerotic stroma characterizes cholangiocarcinoma, and an origin or close interconnection with adjacent canals of Herring may be seen. Except for some of the morphologic types already mentioned, morphology is otherwise not useful for identification of carcinoma of unknown primary.

Immunohistochemistry

The limitations of morphology have fueled the search for markers of differentiation. Since the first major publication reporting cytokeratin phenotype of CK7 and CK20 as discriminatory among different tumor types and sites,⁵⁸ it has become the cornerstone of the panel to evaluate tumors of unknown origin. There are four possible expression patterns: CK7+/CK20+, CK7+/CK20–, CK7–/CK20+ and CK7–/CK20–. One difficulty with interpreting the CK7/CK20 phenotype is that the criteria used to define a tumor as positive for antigen expression have varied significantly among authors.⁵⁸⁻⁶⁰ Some authors have required only 1% of cells while others have required at least 50%. Adding to the difficulties in interpreting the findings is that the antibodies used are different and that there are differences in antigen retrieval techniques and performance of the immunohistochemical studies. The CK7/CK20 phenotype is also influenced by the degree of differentiation and the morphologic subtype. All of these caveats mean that the pathologic interpretation of CK7/CK20 immunohistochemical studies remains subjective, but it remains most effective when based on an algorithmic and probabilistic approach. However, despite these limitations, the CK7/CK20 expression pattern is effective at narrowing down possibilities.

When applying an algorithmic approach to identifying the origin of an adenocarcinoma, the first step involves distinguishing the primary from a metastasis. In the case of cholangiocarcinoma, the diagnosis remains one of exclusion in most cases because its phenotype overlaps with that of many other carcinomas.

Table 3. — Cytokeratin Coexpression Patterns

CK7+/CK20+	CK7-/CK20-	CK7-/CK20+	CK7-/CK20-
Urothelial carcinoma	Breast	Colorectal	Prostate
Pancreas	Lung		Renal cell carcinoma
Biliary tract	Esophagus/stomach		Hepatocellular carcinoma
Cholangiocarcinoma	Pancreas		Adrenal cortical carcinoma
Esophagus/stomach	Biliary		
Mucinous carcinoma (ovarian, colon, mucinous bronchoalveolar)	Cholangiocarcinoma		
	Ovary (nonmucinous)		
	Endometrium		

Cholangiocarcinomas are usually CK7+ and variably express CK20.⁵⁹ Their morphologic expression pattern overlaps with the pattern of many other primary sites.

The most relevant phenotype to the discussion of metastatic adenocarcinoma to the liver is the CK7-/CK20 + phenotype because it is highly characteristic of colorectal primary.⁶¹ The predictive probability of this immunophenotype is 78%.⁶²

Carcinomas coexpressing CK7/CK20 include urothelial carcinoma, metastatic pancreatobiliary carcinoma, and mucinous ovarian carcinoma.^{58,63} Recent publications have shown that mucinous colon carcinoma and mucinous bronchoalveolar carcinoma also express the CK7+/CK20+ phenotype.^{64,67} Morphology can sometimes exclude metastatic urothelial carcinoma since this is not a gland-forming neoplasm; however, its features may overlap with those of poorly differentiated adenocarcinoma. The CK7-/CK20- phenotype is usually typical of prostate, with a probability of 76%.⁶² Other tumors rarely show this phenotype. The CK7+/CK20-subtype is the least specific.⁶³ Lung and breast are two tumors that exclusively have this phenotype, with a probability of 84% and 88%, respectively. However, other tumors can express this phenotype, particularly if CK20 expression is weak or focal. Of note, gastric and esophageal adenocarcinoma have the most variable CK7/CK20 expression pattern.^{58,61} Therefore, these need to be included in the differential diagnosis of any phenotype. Table 3 summarizes the most frequent CK7/CK20 expression patterns for carcinomas of different primary sites.

Other studies can help to further refine the differential diagnosis. Cytokeratin 17 is associated with ampullary and pancreatobiliary carcinomas more often than gastric or esophageal carcinomas.^{68,69} Additional markers help to further refine the identification such as TTF1 (nonmucinous pulmonary adenocarcinomas),^{64,70-74} estrogen receptor protein staining (breast, ovarian, endometrial),^{29,75} GCDFP15 (breast),^{27,76-78} WT1 (ovarian serous tumors),⁷⁹ PSA and PAP (prostate),⁸⁰ thrombomodulin,⁸¹ and uroplakin (urothelial carcinoma).^{80,82} CA125, when used as part of a panel, is useful for identifying ovarian carcinomas.^{83,84} CDX2 is used as a marker of intestinal differentiation and can help to differentiate colorectal, intestinal, or gastric neoplasms

from pancreatobiliary, biliary, ovarian, or pulmonary adenocarcinomas.^{65,85-87}

An older antibody panels consisting of MOC31, keratins, vimentin, B72.3, CA125, Ca19-9, placental alkaline phosphatase, S100 protein, estrogen receptor protein, PSA, thyroglobulin, GCDFP15, and CEA obtained a sensitivity of 67%⁸⁸ for the diagnosis of carcinoma of unknown primary. Another study evaluating GCDFP15, breast cancer antigen 225 (BCA225), B72.3, CA15-3, CEA, CA19-9, CA125, and estrogen receptor showed a sensitivity of 67% for the determination of site of origin.⁸⁹ While individual studies have evaluated the sensitivity and specificity of sets of markers for specific differential diagnoses, the sensitivity of a panel including CK7 and CK20 with some of the above listed antibodies has not been determined across a broad number of cancers in the liver.

Future Directions

Cancer therapy is primarily directed by tumor origin, making correct pathologic diagnosis imperative for proper patient management. As discussed, the number of specific markers available and subjectivity of interpretation are factors that limit standard pathologic practice using morphology and immunohistochemistry. Tumor classification, using high throughput technologies such as microarray and proteomic screening, promise to improve cancer diagnosis and management.

A number of publications have demonstrated the feasibility of using gene expression profiles derived from microarray analysis as an approach to diagnosis.⁹⁰⁻⁹² Proteomic analysis has the potential to yield similar data sets.⁹³

In order for these technologies to be clinically relevant, they must be applicable with FNA and NCB. Recent studies have shown the feasibility of using FNA or NCB specimens for microarray analysis.⁹⁴⁻⁹⁹ In our own experience, FNA of resection specimens obtained over 1 µg of total RNA for microarray analysis.¹⁰⁰ The samples were successfully classified using a tumor classifier derived from resection specimens. Studies evaluating the use of cytology or NCB material for proteomic analysis are limited.¹⁰¹

The problem for any of these technologies is the lack of standardization in specimen collection and preparation. However, these technologies promise to supplement and improve our current standard of practice as adjunctive techniques.

References

1. Abbruzzese JL, Abbruzzese MC, Lenzi R, et al. Analysis of a diagnostic strategy for patients with suspected tumors of unknown origin. *J Clin Oncol*. 1995;13:2094-2103.
2. Axe SR, Erozan YS, Ermatinger SV. Fine-needle aspiration of the liver. A comparison of smear and rinse preparations in the detection of cancer. *Am J Clin Pathol*. 1986;86:281-285.
3. Stewart CJ, Coldewey J, Stewart IS. Comparison of fine needle aspiration cytology and needle core biopsy in the diagnosis of radiologically detected abdominal lesions. *J Clin Pathol*. 2002;55:93-97.
4. Jacobsen GK, Gammelgaard J, Fuglo M. Coarse needle biopsy versus fine needle aspiration biopsy in the diagnosis of focal lesions of the liver: ultrasonically guided needle biopsy in suspected hepatic malignancy. *Acta Cytol*. 1983;27:152-156.
5. Bedenne L, Mottot C, Courtois B, et al. Is the Tru-Cut needle more efficient than the fine needle in the diagnosis of hepatic lesions? Comparative study of 45 echography-guided punctures. *Gastroenterol Clin Biol [French]*. 1990;14:62-66.
6. Livraghi T, Sangalli G, Giordano F, et al. Fine aspiration versus fine cutting needle, and comparison between smear cytology, inclusion cytology and microhistology in abdominal lesions. *Tumori*. 1988;74:361-364.
7. Cochand-Priollet B, Chagnon S, Ferrand J, et al. Comparison of cytologic examination of smears and histologic examination of tissue cores obtained by fine needle aspiration biopsy of the liver. *Acta Cytol*. 1987;31:476-480.
8. Lin BP, Chu JM, Rose RA. Ultrasound guided fine needle biopsy of the liver for cytology and histology. *Australas Radiol*. 1991;35:33-37.
9. Solmi L, Muratori R, Bacchini P, et al. Comparison between echo-guided fine-needle aspiration cytology and microhistology in diagnosing pancreatic masses. *Surg Endosc*. 1992;6:222-224.
10. Nyman RS, Cappelen-Smith J, Brismar J, et al. Yield and complications in ultrasound-guided biopsy of abdominal lesions. Comparison of fine-needle aspiration biopsy and 1.2-mm needle core biopsy using an automated biopsy gun. *Acta Radiol*. 1995;36:485-490.
11. Hugosson CO, Nyman RS, Cappelen-Smith JM, et al. Ultrasound-guided biopsy of abdominal and pelvic lesions in children: a comparison between fine-needle aspiration and 1.2 mm-needle core biopsy. *Pediatr Radiol*. 1999;29:31-36.
12. Moulton JS, Moore PT. Coaxial percutaneous biopsy technique with automated biopsy devices: value in improving accuracy and negative predictive value. *Radiology*. 1993;186:515-522.
13. Miller DA, Carrasco CH, Katz RL, et al. Fine needle aspiration biopsy: the role of immediate cytologic assessment. *AJR Am J Roentgenol*. 1986;147:155-158.
14. Silverman JF, Finley JL, O'Brien KF, et al. Diagnostic accuracy and role of immediate interpretation of fine needle aspiration biopsy specimens from various sites. *Acta Cytol*. 1989;33:791-796.
15. Logrono R, Kurtzy DF, Sprout IA, et al. Multidisciplinary approach to deep-seated lesions requiring radiologically-guided fine-needle aspiration. *Diagn Cytopathol*. 1998;18:338-342.
16. Bell DA, Carr CP, Szyfelbein WM. Fine needle aspiration cytology of focal liver lesions: results obtained with examination of both cytologic and histologic preparations. *Acta Cytol*. 1986;30:397-402.
17. Chiu KW, Chang-Chien CS, Chen L, et al. Ultrasonically-guided needle aspiration with preparation of cell blocks in the diagnosis of liver tumors. *Hepatogastroenterology*. 1994;41:30-33.
18. Kern WH, Haber H. Fine needle aspiration mini biopsies. *Acta Cytol*. 1986;30:403-408.
19. Hahn PF, Eisenberg PJ, Pitman MB, et al. Cytopathologic touch preparations (imprints) from core needle biopsies: accuracy compared with that of fine-needle aspirates. *AJR Am J Roentgenol*. 1995;165:1277-1279.
20. Zardawi IM. Fine needle aspiration cytology vs core biopsy in a rural setting. *Acta Cytol*. 1998;42:883-887.
21. Shak K, Goodman Z, Stocker J, eds. *Tumors of the Liver and Intrahepatic Bile Ducts*. 3rd series. Vol 31. Washington, DC: AFIP; 2001.
22. Dey P, Amir T, Jogai S, et al. Fine-needle aspiration cytology of metastatic transitional cell carcinoma. *Diagn Cytopathol*. 2005;32:226-228.
23. Kaufmann O, Dietel M. Expression of thyroid transcription factor-1 in pulmonary and extrapulmonary small cell carcinomas and other neuroendocrine carcinomas of various primary sites. *Histopathology*. 2000;36:415-420.
24. Pavlidis N, Fizazi K. Cancer of unknown primary (CUP). *Crit Rev Oncol Hematol*. 2005;54:243-250.
25. Varadhachary GR, Abbruzzese JL, Lenzi R. Diagnostic strategies for unknown primary cancer. *Cancer*. 2004;100:1776-1785.
26. Tot T. The role of cytokeratins 20 and 7 and estrogen receptor analysis in separation of metastatic lobular carcinoma of the breast and metastatic signet ring cell carcinoma of the gastrointestinal tract. *APMIS*. 2000;108:467-472.
27. Kaufmann O, Deidesheimer T, Muehlenberg M, et al. Immunohistochemical differentiation of metastatic breast carcinomas from metastatic adenocarcinomas of other common primary sites. *Histopathology*. 1996;29:233-240.
28. Yim H, Jin YM, Shim C, et al. Gastric metastasis of mammary signet ring cell carcinoma: a differential diagnosis with primary gastric signet ring cell carcinoma. *J Korean Med Sci*. 1997;12:256-261.
29. Nash JW, Morrison C, Frankel WL. The utility of estrogen receptor and progesterone receptor immunohistochemistry in the distinction of metastatic breast carcinoma from other tumors in the liver. *Arch Pathol Lab Med*. 2003;127:1591-1595.
30. Pitman MB. Fine needle aspiration biopsy of the liver: principal diagnostic challenges. *Clin Lab Med*. 1998;18:483-506, vi.
31. Lack EE, Mulvihill JJ, Travis WD, et al. Adrenal cortical neoplasms in the pediatric and adolescent age group: clinicopathologic study of 30 cases with emphasis on epidemiological and prognostic factors. *Pathol Annu*. 1992;27(Pt 1):1-53.
32. Serrano R, Rodriguez-Peralto JL, Santos-Briz A, et al. Fine needle aspiration cytology of metastatic hepatic adrenocortical carcinoma mimicking hepatocellular carcinoma: a case report. *Acta Cytol*. 2001;45:768-770.
33. Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. *Br J Biomed Sci*. 2002;59:196-202.
34. Clarkson KS, Sturdge IC, Molyneux AJ. The usefulness of tyrosinase in the immunohistochemical assessment of melanocytic lesions: a comparison of the novel T311 antibody (anti-tyrosinase) with S-100, HMB45, and A103 (anti-melan-A). *J Clin Pathol*. 2001;54:196-200.
35. Dong HY, Harris NL, Preffer FI, et al. Fine-needle aspiration biopsy in the diagnosis and classification of primary and recurrent lymphoma: a retrospective analysis of the utility of cytomorphology and flow cytometry. *Mod Pathol*. 2001;14:472-481.
36. Moriarty AT, Wiersema L, Snyder W, et al. Immunophenotyping of cytologic specimens by flow cytometry. *Diagn Cytopathol*. 1993;9:252-258.
37. Liu K, Mann KP, Vitellas KM, et al. Fine-needle aspiration with flow cytometric immunophenotyping for primary diagnosis of intra-abdominal lymphomas. *Diagn Cytopathol*. 1999;21:98-104.
38. Wieczorek TJ, Faquin WC, Rubin BP, et al. Cytologic diagnosis of gastrointestinal stromal tumor with emphasis on the differential diagnosis with leiomyosarcoma. *Cancer*. 2001;93:276-287.
39. Loy TS, Phillips RW, Linder CL. A103 immunostaining in the diagnosis of adrenal cortical tumors: an immunohistochemical study of 316 cases. *Arch Pathol Lab Med*. 2002;126:170-172.
40. Shin SJ, Hoda RS, Ying L, et al. Diagnostic utility of the monoclonal antibody A103 in fine-needle aspiration biopsies of the adrenal. *Am J Clin Pathol*. 2000;113:295-302.
41. Renshaw AA, Granter SR. A comparison of A103 and inhibin reactivity in adrenal cortical tumors: distinction from hepatocellular carcinoma and renal tumors. *Mod Pathol*. 1998;11:1160-1164.
42. Jalali M, Krishnamurthy S. Comparison of immunomarkers for the identification of adrenocortical cells in cytology specimens. *Diagn Cytopathol*. 2005;33:78-82.
43. Jorda M, De MB, Nadji M. Calretinin and inhibin are useful in separating adrenocortical neoplasms from pheochromocytomas. *Appl Immunohistochem Mol Morphol*. 2002;10:67-70.
44. Zhang PJ, Genega EM, Tomaszewski JE, et al. The role of calretinin, inhibin, melan-A, BCL-2, and C-kit in differentiating adrenal cortical and medullary tumors: an immunohistochemical study. *Mod Pathol*. 2003;16:591-597.
45. Avery AK, Beckstead J, Renshaw AA, et al. Use of antibodies to RCC and CD10 in the differential diagnosis of renal neoplasms. *Am J Surg Pathol*. 2000;24:203-210.
46. Beham A, Ratschek M, Zatloukal K, et al. Immunohistochemical analysis of 42 renal cell carcinomas and one oncocytoma with mono- and polyclonal antibodies against vimentin and cytokeratin. *Verh Dtsch Ges Pathol*. 1989;73:392-395.
47. Medeiros LJ, Gelb AB, Weiss LM. Low-grade renal cell carcinoma: a clinicopathologic study of 53 cases. *Am J Surg Pathol*. 1987;11:633-642.
48. Tretiakova MS, Sahoo S, Takahashi M, et al. Expression of alpha-methylacyl-CoA racemase in papillary renal cell carcinoma. *Am J Surg Pathol*. 2004;28:69-76.
49. Borscheri N, Roessner A, Rocken C. Canalicular immunostaining of nephrilysin (CD10) as a diagnostic marker for hepatocellular carcinomas. *Am J Surg Pathol*. 2001;25:1297-1303.
50. Johnson DE, Herndier BG, Medeiros LJ, et al. The diagnostic utility of the keratin profiles of hepatocellular carcinoma and cholangiocarcinoma. *Am J Surg Pathol*. 1988;12:187-197.
51. Balaton AJ, Nehama-Sibony M, Gotheil C, et al. Distinction between hepatocellular carcinoma, cholangiocarcinoma, and metastatic carcinoma based on immunohistochemical staining for carcinoembryonic antigen and

for cytokeratin 19 on paraffin sections. *J Pathol.* 1988;156:305-310.

52. Porcell AI, De Young BR, Proca DM, et al. Immunohistochemical analysis of hepatocellular and adenocarcinoma in the liver: MOC31 compares favorably with other putative markers. *Mod Pathol.* 2000;13:773-778.

53. Proca DM, Niemann TH, Porcell AI, et al. MOC31 immunoreactivity in primary and metastatic carcinoma of the liver: report of findings and review of other utilized markers. *Appl Immunohistochem Mol Morphol.* 2000;8:120-125.

54. Fan Z, van de Rijn M, Montgomery K, et al. Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. *Mod Pathol.* 2003;16:137-144.

55. Lau SK, Prakash S, Geller SA, et al. Comparative immunohistochemical profile of hepatocellular carcinoma, cholangiocarcinoma, and metastatic adenocarcinoma. *Hum Pathol.* 2002;33:1175-1181.

56. Morrison C, Marsh W Jr, Frankel WL. A comparison of CD10 to pCEA, MOC-31, and hepatocyte for the distinction of malignant tumors in the liver. *Mod Pathol.* 2002;15:1279-1287.

57. Chu PG, Ishizawa S, Wu E, et al. Hepatocyte antigen as a marker of hepatocellular carcinoma: an immunohistochemical comparison to carcinoembryonic antigen, CD10, and alpha-fetoprotein. *Am J Surg Pathol.* 2002;26:978-988.

58. Wang NP, Zee S, Zarbo RJ, et al. Coordinate expressions of cytokeratins 7 and 20 defines unique subsets of carcinomas. *Appl Immunohistochem.* 1995;3:99-107.

59. Maeda T, Kajiyama K, Adachi E, et al. The expression of cytokeratins 7, 19, and 20 in primary and metastatic carcinomas of the liver. *Mod Pathol.* 1996;9:901-909.

60. Maeda T, Takenaka K, Taguchi K, et al. Adenosquamous carcinoma of the liver: clinicopathologic characteristics and cytokeratin profile. *Cancer.* 1997;80:364-371.

61. Tot T. Adenocarcinomas metastatic to the liver: the value of cytokeratins 20 and 7 in the search for unknown primary tumors. *Cancer.* 1999;85:171-177.

62. Tot T. The value of cytokeratins 20 and 7 in discriminating metastatic adenocarcinomas from pleural mesotheliomas. *Cancer.* 2001;92:2727-2732.

63. Tot T, Samii S. The clinical relevance of cytokeratin phenotyping in needle biopsy of liver metastasis. *APMIS.* 2003;111:1075-1082.

64. Lau SK, Desrochers MJ, Luthringer DJ. Expression of thyroid transcription factor-1, cytokeratin 7, and cytokeratin 20 in bronchioloalveolar carcinomas: an immunohistochemical evaluation of 67 cases. *Mod Pathol.* 2002;15:538-542.

65. Saad RS, Cho P, Silverman JF, et al. Usefulness of Cdx2 in separating mucinous bronchioloalveolar adenocarcinoma of the lung from metastatic mucinous colorectal adenocarcinoma. *Am J Clin Pathol.* 2004;122:421-427.

66. Ji H, Isacson C, Seidman JD, et al. Cytokeratins 7 and 20, Dpc4, and MUC5AC in the distinction of metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumors: Dpc4 assists in identifying metastatic pancreatic carcinomas. *Int J Gynecol Pathol.* 2002;21:391-400.

67. Goldstein NS, Thomas M. Mucinous and nonmucinous bronchioloalveolar adenocarcinomas have distinct staining patterns with thyroid transcription factor and cytokeratin 20 antibodies. *Am J Clin Pathol.* 2001;116:319-325.

68. Kim MA, Lee HS, Yang HK, et al. Cytokeratin expression profile in gastric carcinomas. *Hum Pathol.* 2004;35:576-581.

69. Goldstein NS, Bassi D. Cytokeratins 7, 17, and 20 reactivity in pancreatic and ampulla of Vater adenocarcinomas: percentage of positivity and distribution is affected by the cut-point threshold. *Am J Clin Pathol.* 2001;115:695-702.

70. Kaufmann O, Dietel M. Thyroid transcription factor-1 is the superior immunohistochemical marker for pulmonary adenocarcinomas and large cell carcinomas compared to surfactant proteins A and B. *Histopathology.* 2000;36:8-16.

71. Lau SK, Luthringer DJ, Eisen RN. Thyroid transcription factor-1: a review. *Appl Immunohistochem Mol Morphol.* 2002;10:97-102.

72. Chhieng DC, Cangiarella JF, Zakowski MF, et al. Use of thyroid transcription factor 1, PE-10, and cytokeratins 7 and 20 in discriminating between primary lung carcinomas and metastatic lesions in fine-needle aspiration biopsy specimens. *Cancer.* 2001;93:330-336.

73. Simsir A, Wei XJ, Yee H, et al. Differential expression of cytokeratins 7 and 20 and thyroid transcription factor-1 in bronchioloalveolar carcinoma: an immunohistochemical study in fine-needle aspiration biopsy specimens. *Am J Clin Pathol.* 2004;121:350-357.

74. Hecht JL, Pinkus JL, Weinstein LJ, et al. The value of thyroid transcription factor-1 in cytologic preparations as a marker for metastatic adenocarcinoma of lung origin. *Am J Clin Pathol.* 2001;116:483-488.

75. O'Connell FP, Wang HH, Odze RD. Utility of immunohistochemistry in distinguishing primary adenocarcinomas from metastatic breast carcinomas in the gastrointestinal tract. *Arch Pathol Lab Med.* 2005;129:338-347.

76. Lee BH, Hecht JL, Pinkus JL, et al. WT1, estrogen receptor, and progesterone receptor as markers for breast or ovarian primary sites in metastatic adenocarcinoma to body fluids. *Am J Clin Pathol.* 2002;117:745-750.

77. Fiel MI, Cernaianu G, Burstein DE, et al. Value of GCDFP-15 (BRST-2) as a specific immunocytochemical marker for breast carcinoma in cytologic specimens. *Acta Cytol.* 1996;40:637-641.

78. Wick MR, Lillemoe TJ, Copland GT, et al. Gross cystic disease fluid protein-15 as a marker for breast cancer: immunohistochemical analysis of 690 human neoplasms and comparison with alpha-lactalbumin. *Hum Pathol.* 1989;20:281-287.

79. Shimizu M, Toki T, Takagi Y, et al. Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors. *Int J Gynecol Pathol.* 2000;19:158-163.

80. Mhawech P, Uchida T, Pelte MF. Immunohistochemical profile of high-grade urothelial bladder carcinoma and prostate adenocarcinoma. *Hum Pathol.* 2002;33:1136-1140.

81. Parker DC, Folpe AL, Bell J, et al. Potential utility of uroplakin III, thrombomodulin, high molecular weight cytokeratin, and cytokeratin 20 in noninvasive, invasive, and metastatic urothelial (transitional cell) carcinomas. *Am J Surg Pathol.* 2003;27:1-10.

82. Kaufmann O, Volmerig J, Dietel M. Uroplakin III is a highly specific and moderately sensitive immunohistochemical marker for primary and metastatic urothelial carcinomas. *Am J Clin Pathol.* 2000;113:683-687.

83. Legendijk JH, Mullink H, van Diest PJ, et al. Immunohistochemical differentiation between primary adenocarcinomas of the ovary and ovarian metastases of colonic and breast origin: comparison between a statistical and an intuitive approach. *J Clin Pathol.* 1999;52:283-290.

84. Legendijk JH, Mullink H, Van Diest PJ, et al. Tracing the origin of adenocarcinomas with unknown primary using immunohistochemistry: differential diagnosis between colonic and ovarian carcinomas as primary sites. *Hum Pathol.* 1998;29:491-497.

85. Li MK, Folpe AL. CDX-2, a new marker for adenocarcinoma of gastrointestinal origin. *Adv Anat Pathol.* 2004;11:101-105.

86. Raspollini MR, Amunni G, Villanucci A, et al. Utility of CDX-2 in distinguishing between primary and secondary (intestinal) mucinous ovarian carcinoma: an immunohistochemical comparison of 43 cases. *Appl Immunohistochem Mol Morphol.* 2004;12:127-131.

87. Saad RS, Essig DL, Silverman JF, et al. Diagnostic utility of CDX-2 expression in separating metastatic gastrointestinal adenocarcinoma from other metastatic adenocarcinoma in fine-needle aspiration cytology using cell blocks. *Cancer.* 2004;102:168-173.

88. DeYoung BR, Wick MR. Immunohistologic evaluation of metastatic carcinomas of unknown origin: an algorithmic approach. *Semin Diagn Pathol.* 2000;17:184-193.

89. Brown RW, Campagna LB, Dunn JK, et al. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma: a diagnostic adjunct in the determination of primary site. *Am J Clin Pathol.* 1997;107:12-19.

90. Alizadeh AA, Ross DT, Perou CM, et al. Towards a novel classification of human malignancies based on gene expression patterns. *J Pathol.* 2001;195:41-52.

91. Yeang CH, Ramaswamy S, Tamayo P, et al. Molecular classification of multiple tumor types. *Bioinformatics.* 2001;17(suppl 1):S316-S322.

92. Bloom G, Yang IV, Boulware D, et al. Multi-platform, multi-site, microarray-based human tumor classification. *Am J Pathol.* 2004;164:9-16.

93. Alaiya AA, Franzen B, Hagman A, et al. Molecular classification of borderline ovarian tumors using hierarchical cluster analysis of protein expression profiles. *Int J Cancer.* 2002;98:895-899.

94. Ellis M, Davis N, Coop A, et al. Development and validation of a method for using breast core needle biopsies for gene expression microarray analyses. *Clin Cancer Res.* 2002;8:1155-1166.

95. Pusztai L, Ayers M, Stec J, et al. Gene expression profiles obtained from fine-needle aspirations of breast cancer reliably identify routine prognostic markers and reveal large-scale molecular differences between estrogen-negative and estrogen-positive tumors. *Clin Cancer Res.* 2003;9:2406-2415.

96. Sotiriou C, Powles TJ, Dowsett M, et al. Gene expression profiles derived from fine needle aspiration correlate with response to systemic chemotherapy in breast cancer. *Breast Cancer Res.* 2002;4:R3. Epub 2002 Mar 20.

97. Symmans WF, Ayers M, Clark EA, et al. Total RNA yield and microarray gene expression profiles from fine-needle aspiration biopsy and core-needle biopsy samples of breast carcinoma. *Cancer.* 2003;97:2960-2971.

98. Assersohn L, Gangi L, Zhao Y, et al. The feasibility of using fine needle aspiration from primary breast cancers for cDNA microarray analyses. *Clin Cancer Res.* 2002;8:794-801.

99. Dunmire V, Wu C, Symmans WF, et al. Increased yield of total RNA from fine-needle aspirates for use in expression microarray analysis. *Biotechniques.* 2002;33:890-892, 894, 896.

100. Centeno BA, Enkemann SA, Coppola D, et al. Classification of human tumors using gene expression profiles obtained after microarray analysis of fine-needle aspiration biopsy samples. *Cancer.* 2005;105:101-109.

101. Fetsch PA, Simone NL, Bryant-Greenwood PK, et al. Proteomic evaluation of archival cytologic material using SELDI affinity mass spectrometry: potential for diagnostic applications. *Am J Clin Pathol.* 2002;118:870-876.