Background: The simplest definition of de novo intraepidermal epithelioid melanocytic dysplasia (DNIEMD) is that it is a frequent lesion that is neither a dysplastic nevus nor a melanoma in situ. Histologic features include a single-cell pagetoid growth pattern of melanocytes without epidermal melanocytic hyperplasia, stromal alterations, and insufficient architectural changes with significant melanocytic atypia. The biological significance of DNIEMD is unclear, and even the entity itself has been debated.

Objective: To study a large collection of DNIEMDs to better understand the clinicopathologic features of this entity.

Methods: The authors reviewed 263 biopsies from 258 patients diagnosed over approximately a 17-month period. The authors diagnosed DNIEMD when the following histologic findings were present: (1) an intraepidermal proliferation of atypical epithelioid melanocytes; (2) a predominantly pagetoid growth pattern; and (3) no stromal changes such as those seen in dysplastic nevus (lamellar fibroplasia, etc). The authors further graded the lesions as mild, moderate, or severe. Clinical and histopathologic information was recorded when available, with a questionnaire sent to the referring physicians.

Results: Interestingly, 82% of cases were from women, and 71% of lesions were from the lower extremities (upper extremities, 24%). Only in the truncal region did men outnumber women. The age range was from 10 to 84 years, with a mean age of 47 and 52 years for women and men, respectively. The average lesional size was 0.35 cm. In the majority of lesions, the atypia was diagnosed as moderate. In addition to the histology described above, junctional nests were absent or comprised <25% of the lesion. By clinicopathologic correlation, 90% of patients had a Fitzpatrick skin type of I or II, which corresponds to an individual who is very sun sensitive, burns easily, and tans minimally. A significant number of patients had a personal history of dysplastic nevus (68% of those reporting) or a family history or personal history of melanoma (24% each of those reporting).

Conclusions: De novo intraepidermal epithelioid melanocytic dysplasias are relatively common lesions typically found on the lower extremities of women. There is an association with malignant melanoma and dysplastic nevus as well as increased UV exposure.

Reviewer's Comments: I try to review articles discussing melanocytic lesions, as they are some of the most challenging lesions and usually provoke the most angst in dermatopathology. This article highlights another entity within the already complex list of melanocytic lesions. However, this is a lesion that appears to be common and encompasses histologic features that comprise the atypical melanocytic lesions. As the authors indicate, follow-up studies on patients with these lesions will provide usual information on the biologic nature of DNIEMDs. (Reviewer-William A. Kanner, MD).

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Keywords: DNIEMD, Skin, Melanoma, Dysplastic Nevus

Print Tag: Refer to original journal article
The presence of endometrial stroma is a consistent feature of directly sampled mucosal endometriosis on Pap tests.

**Background:** Endometrium directly sampled on a Pap test can mimic neoplastic endocervical cells. This problem is particularly troublesome in patients who have endocervical mucosal endometriosis. Unfortunately, patients with a history of high-grade cervical intraepithelial neoplasia (CIN2+) and adenocarcinoma in situ (AIS) are at increased risk of mucosal endometriosis due to prior LEEP or conization procedures.

**Objective:** To analyze a series of Pap tests from patients with cervical mucosal endometriosis and AIS, and to compare and contrast the diagnostic criteria.

**Methods:** Patients with biopsy-confirmed cervical mucosal endometriosis over a 7-year period with material available for review were included. All Pap tests within the preceding 12 months before the biopsy were evaluated. An additional 12 Pap tests containing AIS from patients with biopsy-proven disease were included for comparison. All Pap tests were reviewed for the presence or absence of endometrial-type glandular cells, endometrial-type stromal cells, apoptotic bodies, and mitotic figures.

**Results:** 21 Pap tests (11 ThinPrep, 10 conventional) from 16 patients with mucosal endometriosis were reviewed. Thirteen (62%) of the Pap tests had lesional cells present on the slide, including both glandular and stromal cells in 10 cases (48%), stromal cells only in 2 cases (10%), and glandular cells only in 1 case (5%). Endometrial stroma predominated in most cases. On conventional smears, the lesional tissue typically formed biphasic gland and stromal aggregates, whereas on ThinPrep slides, the glands and stroma were often in smaller, separate clusters. The original diagnoses on these Pap tests were atypical endocervical cells in 3 cases, atypical squamous cell of undetermined significance in 4 cases, and negative findings in 14 cases. Endometrial stroma was present in 92% of mucosal endometriosis Pap tests versus 8% of AIS cases, which was statistically significant. Apoptotic bodies were present in 15% of mucosal endometriosis cases compared to 58% of AIS cases, which was also statistically significant. Mitotic figures were identified in equal proportions in both endometriosis and AIS cases.

**Conclusions:** The presence of endometrial stroma was a consistent feature of directly sampled mucosal endometriosis. Pathologists should hesitate before making an interpretation of endometriosis on a Pap test in the absence of stroma, and should consider the possibility of a neoplastic glandular lesion.

**Reviewer's Comments:** This well-designed study looks at a particularly difficult area of gynecologic cytology, the difficulty of differentiating neoplastic glandular lesions from their benign mimics. Identification of endometrial stroma can be difficult, particularly on ThinPrep Pap tests, but is a reassuring sign in cases with hyperchromatic crowded glandular groups. (Reviewer-Deborah J. Chute, MD).

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Keywords: Endometriosis, Pap Test, Endocervical Adenocarcinoma In Situ, Atypical Glandular Cells

Print Tag: Refer to original journal article
Is There Value in Measuring CEA or CA 15.3 in Breast Cancer?

**Prospective Evaluation of Carcinoembryonic Antigen (CEA) and Carbohydrate Antigen 15.3 (CA 15.3) in Patients With Primary Locoregional Breast Cancer.**

Molina R, Auge JM, et al:

Clin Chem 2010; 56 (July): 1148-1157

Measuring carcinoembryonic antigen and carbohydrate antigen 15.3 levels may provide useful prognostic information among breast cancer patients.

**Background:** Various breast cancer tumor markers have been measured for >20 years. In patients with either primary or metastatic breast cancer, wide ranges of marker positivity have been reported. Although some studies have documented a relationship between carcinoembryonic antigen (CEA) and carbohydrate antigen 15.3 (CA 15.3) and tumor and tumor size or nodal involvement, other studies have been unable to duplicate these findings.

**Objective:** To determine the prognostic utility of CEA and CA 15.3 as tumor markers in a large cohort of patients with primary locoregional breast cancer.

**Methods:** CEA and CA 15.3 serum levels were prospectively studied in 2062 consecutive patients treated for breast cancer over a 24-year period. Tumor staging (TNM) characteristics included a predominance of T1 or T2 stage tumors. Axillary node involvement was present in 43.8% of patients, while 56.2% were node negative. The 2 predominant tumor types were infiltrating ductal carcinoma (78.4%) and infiltrating lobular carcinoma (6.8%). Treatments included modified radical mastectomy or lumpectomy plus axillary lymph node dissection, with local radiation and adjuvant systemic chemotherapy as indicated. A total of 476 patients experienced relapse during follow-up, with 81 having locoregional recurrence and 395 having metastasis.

**Results:** Elevated CEA levels (defined as >5 µg/L) and elevated CA 15.3 (defined as >30 kU/L) were identified in 12.7% and 19.6% of patients, respectively. In 28% of cases, one or both markers were elevated. The increases in tumor marker concentrations correlated with large tumor size and nodal involvement. By multivariate analysis, CEA, estrogen receptor status, and tumor size were all independent prognostic factors for survival in the node-positive and node-negative groups. CA 15.3 was an independent prognostic factor in the node-negative group only. All patients found to have CEA levels >7.5 µg/L had recurrence during follow-up. Among node-negative patients with T1 tumors, 56.3% were observed when at least 1 of the 2 serum markers was elevated, while only 9.4% of recurrences were observed when neither marker was elevated.

**Conclusions:** Both CEA and CA 15.3 are useful prognostic markers in breast cancer patients. Elevated CEA levels, in particular, are associated with an increased probability of subclinical disease spread.

**Reviewer's Comments:** It is interesting to note that all patients with CEA levels >7.5 µg/L had negative presurgical imaging studies, supporting the value of CEA in identifying a subset of patients with advanced subclinical disease. (Reviewer-T. David Bourne, MD).

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Keywords: CEA, CA 15.3, Prognosis, Breast Cancer

Print Tag: Refer to original journal article
Granular cell tumors of the colon and rectum are uncommon and are generally benign. However, they can recur if incompletely excised.

**Background:** Granular cell tumors are typically benign. When they involve the digestive tract, they most often occur within the esophagus. They appear to be more common in African Americans and are sometimes noted to be multifocal. Only rarely are they identified in the colon and anus.

**Objective:** To review the clinicopathologic features of a large series of colorectal granular cell tumors seen at a referral institution.

**Methods:** The surgical pathology database of a single institution was searched for all cases of colorectal granular cell tumors seen over a 15-year period. The histology of all available cases was reviewed, and clinical follow-up was pursued.

**Results:** There were 26 cases, 23 of which were biopsy material. Patients were 29 to 64 years of age, with an equal number of men and women. Two thirds of the tumors were from white patients. One patient was found to have 3 tumors, whereas all other patients had only a single tumor. Most patients were asymptomatic; however, 3 patients presented with hematochezia, 3 with a change in bowel habits, 2 with Crohn’s disease, 1 with diverticular disease, and 1 with appendicitis. Nearly three fourths of the tumors were found in the right colon, of which 1 involved the appendix. Only 1 tumor was found in the rectum. Lesions were described as small polyps and ranged in size from 0.2 to 1.8 cm. Patients were often noted to have other colorectal polyps at other sites. Two of 19 patients had regrowth or recurrence of the granular cell tumors at 6 months and 1 year. After subsequent polypectomy, these patients remained free of disease. No patients developed metastases. Tumors involved the mucosa, submucosa, or both and were infiltrative or marginated. Tumors were composed of nests and ribbons of granular cells separated by fibrous septa. Although 40% of cases had nuclear atypia, none had mitoses or necrosis. Ulceration was not present. Nearly half the tumors were surrounded by a lymphoplasmacytic cuff. Hyalinization and calcification were sometimes seen. All cases tested were immunoreactive with antibodies to S100 protein.

**Conclusions:** Granular cell tumors of the colon and rectum are rare and are often identified during routine colonoscopy. They are benign and akin to granular cell tumors elsewhere.

**Reviewer's Comments:** Pathologists should keep granular cell tumor in their differential diagnosis for colorectal stromal neoplasia. Although benign, the tumors can recur if incompletely excised. (Reviewer-Eduard B. Stelow, MD).

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Keywords: Granular Cell Tumor, Colon, Rectum, Immunohistochemistry

Print Tag: Refer to original journal article
Does Histologic LSIL Progress?

Histological 'Progression' From Low (LSIL) to High (HSIL) Squamous Intraepithelial Lesion Is an Uncommon Event and an Indication for Quality Assurance Review.

Chen EY, Tran A, et al:

Mod Pathol 2010; 23 (August): 1045-1051

“Progression” of low-grade squamous intraepithelial lesion to high-grade squamous intraepithelial lesion on sequential tissue samples is a rare event and may warrant critical review of all patient materials.

Background: With cervical cytology providing a screening tool, the histologic biopsy generally drives patient treatment and follow-up scheduling. The treatment threshold of cervical intraepithelial neoplasia (CIN) 2 or CIN3 (high-grade squamous intraepithelial lesion [HSIL]) indicates those who should undergo a therapeutic excisional procedure versus CIN1 (low-grade squamous intraepithelial lesion [LSIL]), which indicates those who can be monitored. The colposcopic confirmation and subsequent monitoring of LSIL is primarily to identify the approximately 10% of patients who may harbor HSIL, as indicated by ALTS data. However, another more recent study (albeit with different methodology) determined that approximately 2% of LSIL cases on colposcopic biopsy would have a CIN3 or cancer outcome.

Objective: To determine the incidence of LSIL on colposcopic biopsy “progressing” to HSIL, with a critical review of diagnostic interpretation.

Methods: Consecutive cervical biopsies diagnosed as CIN1 were reviewed to select those with CIN2/3 on subsequent tissue samples (biopsy, LEEP, or cone). In these cases, each biopsy was re-reviewed and classified as LSIL or HSIL, including a “severity” score to subclassify the perceived severity within each diagnostic group: CIN1 (1-2), CIN2 (3-4), and CIN3 (5-6). Those with tissue available were immunostained for p16. Fifty-three additional consecutive biopsies not included in the study parameters were also blindly reviewed for a control set.

Results: 29 cases of LSIL on biopsy with a documented HSIL outcome were identified, representing 11% of the cervical biopsies. Twenty-four cases were available for review, 2 of which were reclassified as CIN2 and excluded from further study. The follow-up ranged from 1 month to 5 years; 38% were confirmed as HSIL, while the remaining 62% were reclassified as LSIL. In the control group of biopsies, 13 were originally diagnosed as HSIL, and 10 were confirmed in the review (77%). The difference between the HSIL confirmation rate of the study group (38%) and the control group (77%) was significant (P =0.024). Staining of p16 was discordant between the initial and outcome samples in 4 of 12 cases available for testing.

Conclusions: With a critical review of diagnostic interpretation, only approximately 3% of colposcopic-confirmed LSIL cases (CIN1) will have an HSIL (CIN2/3) outcome.

Reviewer's Comments: The authors suggest that the low incidence of HSIL outcome after LSIL biopsy may be due to actual progression, initial under-diagnosis of HSIL, and change in HPV type over time or overdiagnosis of HSIL on follow up. (Reviewer-Mary T. Galgano, MD).

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Keywords: Cervical Dysplasia

Print Tag: Refer to original journal article
In addition to p16 and in situ hybridization, PCR-based assays are a valid method of testing for HPV in oropharyngeal carcinomas.

**Background:** Human papillomavirus (HPV)-associated squamous cell carcinoma (SCC) of the oropharynx is a well-defined entity with its own clinicopathologic characteristics. HPV testing is often requested, as these patients may respond well to chemotherapy. The virus has been associated with poorly differentiated tumors yet improved prognosis. The most common viral subtype is HPV-16, found in 82% of cases. While p16 immunostaining is commonly performed as a surrogate marker for HPV infection, there is discussion about whether in situ hybridization (ISH) or polymerase chain reaction (PCR) should be performed for HPV testing.

**Objective:** To evaluate 3 approaches for testing HPV in oropharyngeal cancer specimens.

**Methods:** PCR with generic L1 primers (AGPCR) and with E7 HPV-16 specific primers (E7PCR) was performed on formalin-fixed, paraffin-embedded (FFPE) tissue samples. Immunohistochemical staining for p16 was also performed. Finally, ISH for multiple HPV genotypes (including 16, 18, 31, and 33) was performed. Histologic grading was reported as well, moderate, or poorly differentiated. For data analysis, the maximum positive rate (MPR) was defined by positivity for either or both of the HPV PCR studies.

**Results:** The sensitivities of E7PCR and AGPCR were approximately 72% and 90%, respectively, compared to the MPR. Regarding the positive AGPCR cases, 82% were HPV-16, 1% of cases were HPV-58, and 17% were positive but it was not possible to determine the subtype. Fourteen cases could be analyzed unequivocally by ISH and AGPCR, and all cases were concordant. All cases that were positive for HPV were strongly positive for p16 (100% sensitivity), yet the specificity was only 38% relative to the MPR. Finally, there was no correlation between HPV status and reported tumor differentiation.

**Conclusions:** PCR-based HPV tests have an additional advantage of being type specific. Studies have indicated that one possible limitation is the sensitivity since primers targeting the L1 regions may miss sequences that are genomically integrated and have lost the capsid-encoding sequences. However, this study showed a sensitivity of 90% using MPR, which is impressive given the suboptimal DNA quality of FFPE tissues. Further E7 positivity was not as sensitive relative to the MPR (72.5%), arguing against loss of L1 HPV sequences in a significant number of HPV-16+ cases. Thus, the capsid-encoding region (L1) is lost in only a minority of cases and does not appear to significantly influence the detection rate of HPV. In summary, the authors show that PCR testing for HPV on FFPE tissues is a valid method for testing oropharyngeal carcinomas.

**Reviewer’s Comments:** The partnership between H&E, immunohistochemistry, and molecular diagnostics continues to grow. While there is still debate about testing for HPV type in such specimens with PCR, it is clear that p16 positivity is a clinically useful parameter correlating with improved survival in some patients with oropharyngeal cancers. (Reviewer-Stacey E. Mills, MD).

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Keywords: PCR, HPV, Oropharyngeal Carcinoma

Print Tag: Refer to original journal article
Common variable immunodeficiency is a heterogeneous disease that involves many areas of pathology and many medical specialties.

**Background:** Common variable immunodeficiency (CVID) is a much discussed and poorly understood disease. It is widely discussed because it is the most common primary immune deficiency, with a prevalence of 1 in 25,000 to 50,000 in the Caucasian population. The challenges associated with the diagnosis and management of CVID are manifested in significant delays in diagnosis (6 to 8 years) and misuse of the diagnostic criteria. Most patients are diagnosed between the ages of 20 and 40 years. CVID is defined as a genetic immune defect with decreased levels of IgG, IgA, and/or IgM, with poor to absent antibody production (demonstrated by lack of response to ≥2 protein vaccines), excluding other causes of hypogammaglobulinemia.

**Objective:** To discuss the management and complications of patients with CVID, with special emphasis on pathology.

**Results:** The respiratory tract is most frequently involved, and the resulting pneumonia is often attributed to *Streptococcus pneumoniae*, *Haemophilus influenzae*, or mycoplasma species. A significant number of CVID patients (8% to 22%) will have complications of granulomatous disease (either localized or systemic). Although the lungs, lymph nodes, and spleen are the most common sites, many other organs have also been reported to be involved. Histologically, the granulomas are variously well formed and noncaseating. Organisms are rarely found. Patients with granulomatous disease appear to be at greater risk for autoimmune disease, such as immune thrombocytopenia purpura or autoimmune hemolytic anemia. Occasionally, there is an intense lymphoid infiltrate accompanying the granulomas in the lung; this is termed lymphocytic interstitial lung disease and represents a poor prognostic factor, along with granulomatous disease. As might be anticipated, the incidence of malignancy is overall increased in CVID, attributed primarily to gastric cancer and non-Hodgkin lymphoma. The lymphomas are usually of the B-cell type. Regarding gastrointestinal (GI) disease, the main complication is transient or persistent diarrhea, with *Giardia lamblia* being the most common organism in infectious cases. Inflammatory bowel disease also represents a significant problem in approximately 19% to 32% of CVID patients. GI biopsies may demonstrate increased intraepithelial lymphocytes and (especially in the small intestine) features of celiac sprue, with a lack of plasma cells also being noteworthy.

**Conclusions:** CVID is a challenging and complex disease that involves many medical specialties. The outlook of patients has improved with immunoglobulin replacement therapy and more effective antibiotic regimens. The main areas of concern now have to do with morbidities associated with inflammation and immune dysregulation.

**Reviewer's Comments:** I wanted to review this article because it highlights a disease that we are often involved with in many aspects, such as the laboratory work-up or in various tissue specimens. Although many of the pathologic features that can be present in tissue specimens are general, such as granulomas or inflammation, with good clinical correlation, such findings could suggest CVID. (Reviewer-William A. Kanner, MD).

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Keywords: Common Variable Immune Deficiency, Treatment

Print Tag: Refer to original journal article
Increased cytotoxic tumor-infiltrating lymphocytes in colon cancer resections are associated with improved survival.

**Background:** Colon cancer is one of the leading causes of cancer death. The most reliable predictor of prognosis is stage. However, some patients with lower-stage cancers may benefit from adjuvant chemotherapy, so additional markers of prognosis are constantly under review. Tumor-infiltrating lymphocytes (TILs) were first identified in association with microsatellite instability (MSI) and the hereditary nonpolyposis colorectal cancer syndrome. Patients with MSI-high colon tumors frequently had TILs, and these patients typically had a better prognosis than those with MSI-low tumors. Recently, research has suggested that the better prognosis may be related to the altered immune response (increased TILs) in these patients.

**Objective:** To evaluate the prognostic impact of TILs in patients with stage II colon cancer with respect to overall survival (OS) and disease-free survival (DFS).

**Methods:** Patients who underwent resection with curative intent for stage II colon carcinoma over a 10-year period were randomly selected for inclusion. Cases with insufficient material for pathologic review or lacking follow-up were excluded. Immunohistochemistry was performed on a section of tumor from each case with antibodies to CD3, CD45RO, CD25, and FOXP3. The number of TILs positive for each immunohistochemical marker was counted using a computerized image analysis system, and the mean number of cells per 40x high power field (HPF) was calculated. TILs were classified as low density if the number/HPF was below the mean of all cases and high density if the number/HPF was above the mean for all cases.

**Results:** 87 patients who met the study criteria were randomly selected. TILs were detected within cancer epithelium and in cancer-associated stroma. The median duration of follow-up was 125 months. The 5-year OS and DFS rates were 89% and 82%, respectively, for all patients. On univariate analysis, high-density TILs positive for CD3, CD45RO, CD25, and FOXP3 were significantly associated with improved DFS. The estimated 5-year OS for patients with a high density of CD45RO and FOXP3 positive TILs was 100% compared to 79% and 78% for patients with low-density TILs. On multivariate analysis, lymphovascular invasion, high density of CD45RO positive TILs, and high density of FOXP3 positive TILs remained significant predictors of OS and DFS.

**Conclusions:** A high density of cytotoxic TILs in colon cancer is associated with improved DFS and OS independent of other conventional pathologic markers. Evaluation for TILs may identify a subset of patients with stage II colon cancer who could benefit from adjuvant chemotherapy.

**Reviewer's Comments:** This study highlights some interesting new directions in the field of anti-tumor immune response and its intersection with pathology. It would be useful to know if the MSI status of these tumors was also tested, and whether it correlated with outcome. (Reviewer-Deborah J. Chute, MD).

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**Keywords:** Colon Cancer, FOXP3, Survival, Tumor-Infiltrating Lymphocytes, CD45RO

**Print Tag:** Refer to original journal article
In human leukocyte antigen-sensitized kidney recipients, high-strength donor-specific antibodies (DSA) class I level alone or low-strength DSA class I level in combination with the Luminex crossmatch provides the best prediction of antibody-mediated rejection.

**Background:** Antibody-mediated rejection of solid-organ allografts is associated with the presence of donor-specific antibodies (DSA) directed against human leukocyte antigens (HLA). There have been 3 generations of crossmatch tests to detect DSA: (1) the original complement-dependent cytotoxicity crossmatch; (2) the more sensitive flow cytometry crossmatch; and (3) the more recently developed solid-phase Luminex crossmatch, which is able to detect both complement binding and non-complement binding anti-HLA antibodies using fluorescent-labeled beads. In contrast to the older-generation complement-dependent or flow cytometric crossmatches, the newer antigen bead assays are cell membrane independent and do not require intact lymphocyte surfaces for testing.

**Objective:** To determine the prognostic impact of pretransplant DSA class and strength as detected by newer antigen bead technology, and to compare this method with previous crossmatch testing methods.

**Methods:** 155 living donor kidney recipients were screened for the presence of pretransplant anti-HLA antibodies. Of these recipients, 37 patients were found to be HLA sensitized. Flow cytometric crossmatches, a single antigen bead test, and the newer Luminex crossmatch were all retrospectively performed on these 37 samples. The results were then analyzed and correlated with the presence of concomitant antibody-mediated rejection as well as kidney function. All clinically suspected cases of rejection were confirmed with needle core kidney biopsies, which included C4d determinations.

**Results:** The authors determined that a mean fluorescence intensity level of 900 had a sensitivity of 75% and a specificity of 90% for antibody-mediated rejection in patients who had pretransplant DSA against class I HLA. The presence of DSA against class II antigens was not predictive for antibody-mediated rejection. The specificity for antibody-mediated rejection increased to 100% as the strength of the DSA class I level increased. The authors also found that the Luminex assay had greater accuracy for DSA detection compared to the flow cytometric crossmatch. Finally, regardless of the crossmatch method, the presence of DSA class I values >900 (mean) fluorescent intensity units was associated with greatly increased specificity for antibody-mediated rejection.

**Conclusions:** In HLA-sensitized kidney recipients, high-strength DSA class I level alone or low-strength DSA class I level in combination with the Luminex crossmatch provides the best prediction of antibody-mediated rejection.

**Reviewer’s Comments:** This article highlights the value of DSA determinations in kidney transplant patients suspected of having clinical rejection. From the pathologist’s perspective, the knowledge of DSA levels may help direct a diligent search for the light microscopic and immunofluorescence findings that may support antibody-mediated rejection. (Reviewer-T. David Bourne, MD).

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Keywords: Allograft Rejection, Kidney Transplant, Donor-Specific Antibodies

Print Tag: Refer to original journal article
The use of p16 immunohistochemistry is helpful for the diagnosis of cervical intraepithelial neoplasia grade 2.

**Background:** Cervical cancer prevention has been successful in areas in which screening, colposcopic evaluation and biopsy, and lesional ablation have been effectively instituted. The identification of precancerous lesions suitable for ablation relies on a number of factors, including the recognition by pathologists of histologically precancerous lesions and distinction of these lesions from benign dysplasia that is most likely to regress. In general, this means the distinction of cervical intraepithelial neoplasia (CIN) grade 1 from CIN2 and CIN3, now believed to represent 2 different diseases rather than a simple spectrum of disease. CIN2, however, is still considered an equivocal diagnosis by some, as it is more likely to regress than CIN3 and is the least reproducible of CIN interpretations.

**Objective:** To investigate the use of biomarkers for a more reproducible diagnosis of CIN2 and CIN3 and their distinction from other lesions.

**Methods:** All cervical biopsies seen over 14 months at a single institution were evaluated. The original diagnosis was recorded, and cases were then reviewed by 2 or 3 pathologists for a "consensus" diagnosis. Pap test results and follow-up histology results were recorded. Immunohistochemistry was performed with antibodies to p16, ki67, and L1 capsid protein. p16 staining was scored based on strength and continuity; ki67 staining was scored based on continuity and location; and L1 staining was considered "positive" or "negative."

**Results:** There were 1455 cervical biopsies, and the mean patient age was 26 years. Twelve pathologists were responsible for the original diagnoses. There was a 74% raw agreement between the original diagnoses and the consensus diagnoses, and the kappa was 0.59. Consensus review tended to render more severe diagnoses than the original diagnoses. p16 and ki67 staining directly correlated, whereas their staining indirectly correlated with L1 staining. Intense and diffuse p16 staining correlated best with CIN2 and CIN3 consensus diagnoses. Nearly 86% of consensus CIN2 or CIN3 diagnoses had strong and diffuse p16 immunoreactivity. p16 staining was more sensitive for the identification of CIN2 and CIN3 than the original pathologist interpretations. p16 staining was directly associated with more severe follow-up diagnoses after a consensus diagnosis of CIN1 or CIN2. Agreement regarding the interpretation of strong and diffuse p16 staining was nearly perfect.

**Conclusions:** p16 immunostaining provides a useful and reliable adjunct for the diagnosis of precancerous cervical lesions.

**Reviewer's Comments:** This well-written paper illustrates the problems with the histologic assessment of HPV-related cervical lesions, especially the diagnosis of CIN2. As evidenced by their data, some pathologists diagnose CIN2 much less frequently than others. (Reviewer-Edward B. Stelow, MD).

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Keywords: Dysplasia, Biomarker, p16, ki67

Print Tag: Refer to original journal article
Preop Biomarkers for Ovarian Masses -- What Works Best?

Serum Biomarker Panels for the Discrimination of Benign From Malignant Cases in Patients With an Adnexal Mass.
Nolen B, Velikokhatnaya L, et al:
Gynecol Oncol 2010; 117 (June): 440-445

CA-125 combined with HE4 provides a discriminating screening method for the preoperative assessment of women with an adnexal mass.

**Background:** The incidence of an adnexal abnormality is approximately 7%, and as many as 5% to 10% of women may undergo prophylactic surgery due to the concern of ovarian carcinoma. Many of these surgeries may be unnecessary since the lifetime incidence of ovarian carcinoma is only 1.4%. Given the aggressiveness of the disease, no screening protocol has provided a safe alternative to surgical exploration. CA-125 has been widely used as a potential biomarker for ovarian carcinoma, but it has poor sensitivity and specificity when used alone. CA-125 in combination with HE4 has recently been proposed as a better discriminating test in assessing the risk of malignancy of adnexal tumors.

**Objective:** To evaluate circulating proteins in women with an adnexal mass compared to the proposed combination of CA-125/HE4.

**Methods:** Serum samples were collected from women with adnexal masses that were subsequently determined to be either benign or ovarian carcinoma (early and late stage). Patients determined to have inflammatory conditions were excluded from the analysis. These samples were evaluated for 65 potential biomarkers with results correlated to outcome.

**Results:** Most of the proposed markers were significantly different between the benign and malignant cohorts, but HE4 and CA-125 were individually the greatest discriminators. CA-125 performed the best in differentiating benign from early stage carcinoma, while HE4 performed the best in differentiating benign from late-stage carcinoma. In combination, the performance improved compared to either marker alone, and no other 2-marker combination exceeded CA-125/HE4. This provides a sensitivity of 74.2% and a specificity of 85% for early stage carcinoma versus a sensitivity of 91.7% for late-stage carcinoma and 83% for the combined group. On multivariate analysis, 3- and 4-marker panels performed marginally better, but always included the CA-125/HE4 combination. In validation studies, none of the 3- or 4-marker panels performed better than CA-125/HE4. In a specific premenopausal cohort, the addition of EGFR did significantly improve the performance of the 2-marker panel.

**Conclusions:** With an extensive search and validation of potential biomarkers in isolation and combination, the performance of CA-125/HE4 provides the best discriminating test for determining benign from malignant ovarian masses (both early and late stage). In premenopausal women, adding EGFR to the panel significantly improves the test performance.

**Reviewer’s Comments:** This extensive screen of potential serum preoperative biomarkers for women with an adnexal mass seems to have validated the use of CA-125/HE4. (Reviewer-Mary T. Galgano, MD).

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Keywords: Serum Biomarkers

Print Tag: Refer to original journal article
Survivors of childhood cancer are at a greatly increased risk for the development of a second malignancy compared to the general public.

**Background:** 80% of children who develop cancer now survive at least 5 years. As this survival rate has improved over the past 4 decades, we have become more aware of the significant incidence of secondary neoplasms. Risks for the development of these tumors include host factors, the original diseases, and treatment regimens. The Childhood Cancer Survival Study (CCSS) now includes >20 years of data for patients who have survived >5 years after the diagnosis of their childhood cancers.

**Objective:** To review subsequent neoplasia that has developed in this cohort.

**Methods:** The CCSS includes patients who were diagnosed with malignancy between 1970 and 1985, who were <21 years old when the malignancies were diagnosed, and who survived at least 5 years after their diagnoses. Original diagnoses, subsequent diagnoses, and treatment regimens were recorded.

**Results:** There were >14,000 patients. Approximately 1400 patients developed subsequent neoplasia, and 2700 subsequent neoplasms were reported. Of these, 802 neoplasms were malignancies other than skin cancers. Also, there were 159 benign meningiomas, 168 other benign neoplasms, and >1500 skin cancers. Of the 1400 patients who developed neoplasms, more than half developed a malignancy that was not a skin cancer. Almost 10% of those who developed a second malignancy also developed a third malignancy. Breast cancer was the most common second malignancy if skin cancer was excluded, and Hodgkin lymphoma was the most common primary malignancy linked to the development of a subsequent malignancy. The 30-year cumulative incidence for the development of a non-skin cancer malignancy was approximately 8%. Patients with Hodgkin lymphoma had an 18% cumulative incidence of second malignancy. Patients with medulloblastoma had the greatest risk for the development of meningioma with a cumulative incidence of 16%. Patients who received radiation were at a higher risk for the development of subsequent neoplasia than those who did not. The 5-year cumulative incidence of breast cancer was 5% among survivors. Survivors had 6 times the risk of developing cancer compared to subjects who had not had childhood cancer. Specifically, survivors were 10 times more likely to develop breast cancer, 11 times more likely to develop thyroid cancer, 19 times more likely to develop bone cancer, 8 times more likely to develop sarcoma, 7 times more likely to develop kidney cancer, and 11 times more likely to develop head and neck cancer. Risk reduction does not occur with increased survival times.

**Conclusions:** Patients who survive a childhood malignancy are at increased risk for the development of other cancers.

**Reviewer's Comments:** Data regarding second malignancies in cancer survivors are important. Long-term modifications in treatment regimens may help lower some of the risks. (Reviewer-Eduard B. Stelow, MD).
In Adenocarcinoma of Small Intestine, Is Proximal Location Better?

Adenocarcinoma of the Small Intestine: A Multi-Institutional Study of 197 Surgically Resected Cases.
Chang H-K, Yu E, et al:

Hum Pathol 2010; 41 (August): 1087-1096

Small intestinal adenocarcinomas located in the distal small bowel have a worse prognosis than adenocarcinomas located in the duodenum.

**Background:** Despite the large size of the small intestine, it accounts for only 5% of all malignant neoplasms of the gastrointestinal tract. The most common small intestinal malignant neoplasm is adenocarcinoma. There is limited knowledge regarding the clinicopathologic characteristics of small intestinal adenocarcinoma (SIAC) because it is so infrequent.

**Objective:** To review the clinicopathologic characteristics of SIACs from 22 institutions and their associations with patient survival.

**Methods:** Surgically resected SIACs from 22 institutions in South Korea were included. SIACs were defined as carcinomas originating in the mucosa of the small intestine (duodenum, jejunum and ileum). Carcinomas extending from other organs or without mucosal involvement (possible metastasis) were excluded. The patients' medical records were reviewed for clinical data, including additional therapy, other malignancies, and survival status. The presence or absence of disease conditions that predispose to SIAC were recorded (eg, Crohn's disease, familial adenomatous polyposis [FAP], hereditary nonpolyposis colon cancer [HNPCC], or Peutz-Jeghers syndrome). Pathologic features were recorded, including tumor location, tumor stage, lymph node status, and accompanying sporadic adenomas.

**Results:** 197 patients with SIAC were included in the study; the mean patient age was 59 years. The majority of tumors were located in the duodenum (55%), with tumors in the jejunum and ileum being less common (30% and 15%, respectively). Most tumors presented at advanced stage, either T3 (32%) or T4 (57%). Regional lymph node metastasis was identified in 51% of cases. Retroperitoneal seeding was seen in 7% of cases. Predisposing conditions were found in 12% of patients; this was most commonly sporadic adenomas, followed by Peutz-Jeghers syndrome and Crohn's disease. No cases of FAP or HNPCC were observed. The median follow-up was 39 months, and the overall 5-year survival rate was 41%. Tumors present in the duodenum were more likely to be higher stage (pT4) and have pancreas invasion. Distal tumors were more likely to have retroperitoneal seeding and a significantly worse 5-year survival (24 vs 74 months). On multivariate analysis, positive lymph node status and location in the distal small bowel (jejenum or ileum) were significant predictors of worse 5-year survival. Tumor stage (pT) did a poor job of stratifying patient outcome.

**Conclusions:** Small intestinal adenocarcinomas are frequently diagnosed at an advanced stage. Tumors located in the distal small bowel have a worse prognosis than proximal tumors, despite a lower pT stage.

**Reviewer's Comments:** It is unfortunate that there are no current good strategies to detect small intestinal adenocarcinomas at an early stage. The paradox of pT3 distal tumors having a worse prognosis than proximal pT4 tumors is unclear, but perhaps pancreatic invasion (one criterion for pT4b tumor stage) is less predictive of poor outcome than penetration through the visceral peritoneum (one criterion for pT4a). (Reviewer-Deborah J. Chute, MD).

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Keywords: Small Intestine, Adenocarcinoma, Adenoma, Survival, Prognosis

Print Tag: Refer to original journal article
As many as 19% of blood collection tubes will test positive for *Aspergillus* contamination when using PCR assays.

**Background:** Invasive aspergillosis is on the rise among immunocompromised patients. A rapid and sensitive diagnostic test to identify this infection is needed, as fungal culture can take up to 2 weeks. Polymerase chain reaction (PCR)-based detection of *Aspergillus* DNA is both rapid and sensitive, but *Aspergillus* is ubiquitous in the environment and could easily contaminate materials. Because of the side effects of anti-fungal medications, false-positive results can have serious patient consequences.

**Objective:** To investigate the possibility of *Aspergillus* contamination in various blood collection tubes.

**Methods:** >500 tubes from a variety of collection vessels were tested, including BD Vacutainer blood tubes with and without additives. A standard fungal culture was performed on each tube by adding sterile saline to the vessel, agitating it, and spreading an aliquot on Sabouraud dextrose agar, which was incubated at 30°C for 2 weeks. For PCR detection, sterile water was similarly added to each vessel, agitated, and then used for DNA extraction. Each sample was tested using an *Aspergillus* targeted molecular beacon assay, and positive results were confirmed using a second TaqMan PCR assay specific for *Aspergillus fumigatus* only. Very strict controls were used at multiple points of the assay to detect possible contamination of the PCR reactions.

**Results:** No fungal colonies grew from any vessels tested. The initial PCR assay was positive for *Aspergillus* DNA in 9.8% of all vials tested. The second *A. fumigatus*-specific PCR assay was confirmatory in 96% of positive cases. Of whole-blood collection tubes, up to 19% were contaminated, while serum blood collection tubes were contaminated in up to 15% of vials. Five of the positive PCR signals were strong, suggesting the assay would still be positive even after dilution with larger volumes of blood.

**Conclusions:** Using PCR techniques, up to 19% of blood collection tubes are contaminated with *Aspergillus* spp. DNA, although the fungal elements were most likely dead. Any attempt to use real-time PCR to detect clinically relevant *Aspergillus* spp. in blood collection samples will be hampered by this ubiquitous contamination.

**Reviewer's Comments:** This study was well designed and had rigorous environmental and PCR controls to prevent contamination of the samples. It shows the importance of knowing about potential contaminants before implementing nucleic acid amplification techniques for clinical use. (Reviewer-Deborah J. Chute, MD).
Focal nodular hyperplasia is a heterogenous lesion with some evidence of clonality and no evidence of TP53 mutations.

Background: Focal nodular hyperplasia (FNH) of the liver usually occurs in young to middle-age females in the setting of a non-cirrhotic liver. The gross description is classically that of a well-demarcated mass with a "fibrous scar." Microscopically, this "scar" is comprised of a fibrovascular zone with hypertrophied arteries. The surrounding hepatocytes are cytologically bland and may show some degenerative changes. Importantly, portal structures are seen in contrast to hepatic adenoma. Although considered a benign reactive lesion, the true pathogenesis and biologic nature is unclear. One proposal is that FNH represents a reaction to a local arterial malformation.

Objective: To analyze a number of cases of FNH for clonality and TP53 mutation status.

Methods: 15 cases of FNH from female patients were identified and studied. Laser-capture microdissection was performed (600 to 1000 cells) with normal tissue being taken for control purposes. For clonality determination in the female patients, X-chromosome inactivation analysis was performed using the androgen receptor gene locus. TP53 gene mutation analysis was performed using direct DNA sequencing for point mutations. Statistical analysis was then performed.

Results: The mean age of the 15 female patients was 36 years, and the mean size of the lesion was 5.1 cm. No patients had a history of viral infection or cirrhosis, and all patients were alive with no evidence of recurrence on follow-up. By clonality, 13 cases were informative; of these, 4 (31%) demonstrated a nonrandom X-chromosome inactivation pattern. There was no association with clonality and other clinicopathologic parameters such as age or nodule size. All 15 cases were negative for mutations in TP53.

Conclusions: It continues to be controversial whether FNH is a benign neoplasm or simply a reactive lesion. Clonality studies have shown varied results, and this trial is no different. However, taken together, there is evidence that this is a neoplastic process in some cases. TP53 mutation that is associated with many tumors is not present in FNH and may be used to rule out this diagnosis.

Reviewer's Comments: Although this lesion typically does not cause a diagnostic dilemma and is a favorite for resident and faculty case presentations, this article is appealing in that the authors "dig deeper" into this lesion to inquire into the fundamental nature of FNH. (Reviewer-William A. Kanner, MD).

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Keywords: Liver, Focal Nodular Hyperplasia

Print Tag: Refer to original journal article
Elastic Fiber Pattern in Regressing Melanoma

Elastic Fiber Pattern in Regressing Melanoma: A Histochemical and Immunohistochemical Study.
Kamino H, Tam S, et al:

J Cutan Pathol 2010; 37 (July): 723-739

The pattern of elastin fibers helps differentiate between regression and scarring fibrosis.

**Background:** Studies have shown that at least partial regression occurs in anywhere from 10% to 35% of melanomas, while complete regression occurs much less commonly. While the prognostic meaning of regression is not clear, it may be associated with a worse prognosis for various reasons. Usually identification of regression is not challenging; however, situations that can be problematic include scarring fibrosis from a previous procedure, trauma, ruptured cyst, or hair follicle.

**Objective:** To identify changes and patterns of elastic fibers in late-stage regression of melanoma compared to melanomas with scarring fibrosis from previous procedures.

**Methods:** The authors studied 33 cases of primary invasive melanoma with late-stage regression. Additionally, 10 cases of invasive melanoma with a prior procedure (ie, biopsy) done 2 weeks to 2 months earlier were used for comparison. In addition to H&E, a Verhoeff's elastic van Gieson stain and immunohistochemistry for elastin was performed.

**Results:** In the adjacent normal skin, the elastin network demonstrated thin fibers in the papillary dermis forming a superficial network perpendicular to the surface. The deeper dermis demonstrated coarser fibers that were oriented parallel to the surface. Within the areas of melanoma, elastic fibers were markedly decreased to absent; in all cases, there was a continuous layer of elastic fibers from the papillary dermis that had been pushed down to the base of the invasive melanoma. In the fibrotic areas of the regressed melanoma cases, there was marked loss of elastic fibers. Interestingly, at the base of the fibrotic area there existed the same continuous layer of compressed thin fibers, presumably from where the invasive melanoma had been before regression. In cases in which there was melanoma with scar from a previous surgery, elastic fibers were again markedly decreased in the area of scarring. At the base of the scar were thick, coarse, and wavy elastic fibers, characteristic to those that would have been present at that level in the dermis before the procedure. No compressed layer of thin elastic fibers was present.

**Conclusions:** In difficult cases of melanoma, especially those in which a prior procedure has been done, staining for elastin helps differentiate between the later fibrosing stage of regressed melanoma and scarring fibrosis.

**Reviewer's Comments:** This article describes a relatively simple and straightforward way of delineating regression from scar tissue in cases in which the difference is very significant. The authors noted that the immunohistochemical stain for elastin performed better than the traditional stain. It is important to understand that regression does not destroy the elastin fibers, but rather they are "pushed down" by the invasive melanoma prior to regression. (Reviewer-William A. Kanner, MD).

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Keywords: Melanoma, Scar, Regression, Elastin Fibers

Print Tag: Refer to original journal article
Why Weigh the Prostate Gland?

*The Weight of the Prostate Gland Is an Excellent Surrogate for Gland Volume.*

Varma M, Morgan JM:

Histopathology 2010; 57 (July): 55-58

Measuring the weight of the prostate gland (without the seminal vesicles) provides a closer approximation of gland volume than calculating the volume from height, width, and length measurements.

**Background:** Formulae for calculating the volume of prostate glands are based on the product of the height, length, and width multiplied by an additional factor (most commonly pi/6), which is derived from the assumption that the gland is ellipsoid. Radiologists and urologists use this calculation to estimate gland volumes from imaging or ultrasound procedures in order to determine a patient’s suitability for brachytherapy or to provide PSA density estimates (PSA per unit prostate volume). The accurate assessment of prostate gland volume is best performed using water displacement measurements, which are impractical in every day practice.

**Objective:** To prospectively evaluate the relationship between prostate weight (g) and volume (cc), and to determine the accuracy of calculated gland volume compared to true gland volume as measured using water displacement methods.

**Methods:** For the study, 20 Radical prostatectomy specimens were assessed. The weight (g) of each prostate gland was measured using an electronic scale (after seminal vesicles had been removed). Determination of the true gland volume was then obtained by measuring water displacement (cc). Gland height, length, and width measurements were made, and the calculated prostate volume was determined as the product of these 3 dimensions multiplied by pi/6. Paired t-tests and linear regression analysis were used to determine the relationship of gland weight to calculated volume and true gland volume.

**Results:** Regression analysis showed that the weight of the prostate (g) provided an approximation ranging from -5.4% to +5.6% of the true gland volume (cc), whereas the calculated volume provided an approximation ranging from -52.8% to +5.0% of the true gland volume (cc).

**Conclusions:** The prostate gland weight (g) provides a more accurate estimate of gland volume (cc) than calculations using gland height, length, and width measurements, which tend to underestimate the true gland volume.

**Reviewer’s Comments:** At our institution, we typically weigh all radical prostatectomy specimens and provide approximate height, width, and length measurements in the gross report. Unlike our radiology or urology colleagues, we do not routinely calculate the gland volume using the formula cited by the authors (height x width x length x pi/6). This practice seems justified, given that the measured weight appears to provide an even better approximation of gland volume than the calculated estimate. (Reviewer-T. David Bourne, MD).

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Keywords: Prostate Gland, Volume, Weight

Print Tag: Refer to original journal article
Update on New Fixative RCL2

Fixation of Brain Tumor Biopsy Specimens with RCL2 Results in Well-Preserved Histomorphology, Immunohistochemistry and Nucleic Acids.

Preusser M, Plumer S, et al:

Brain Pathol 2010; April 14 (epub ahead of print):

RCL2 fixation shows improved nucleic acid preservation and comparable histological detail and immunohistochemical staining quality compared to formalin fixation.

**Background:** Although buffered formalin is the most widely used fixative in most anatomic pathology laboratories, other fixatives may offer certain benefits in terms of preserving DNA or RNA quality. RCL2 is an alcohol-based fixative that contains acetic acid and ethanol, and its use has been described in the processing of thyroid and breast tumors.

**Objective:** (1) To analyze the effect of RCL2 fixation on histology, immunohistochemistry, and nucleic acid quality, and (2) to compare RCL2 fixation and formalin fixation in brain tumor biopsy specimens.

**Methods:** The study included brain tumor biopsy samples from 49 patients. Each sample was collected and sent unfixed from the operating room directly to the laboratory for examination, formalin fixation, processing, paraffin embedding, and H&E staining. A portion of each fresh sample was placed in RCL2 solution at the time of collection, and this RCL2-fixed tissue was also submitted for routine processing and paraffin embedding. An immunohistochemical panel was performed on all formalin-fixed paraffin embedded (FFPE) and RCL2 fixed and embedded (RCLPE) samples using antibodies directed against GFAP, S-100, vimentin, EMA, synaptophysin, NeuN, Map-2, and cytokeratin, among others. DNA and RNA extractions were performed with testing of various gene concentrations to assess nucleic acid quality.

**Results:** Compared to FFPE samples, RCLPE specimens showed comparable histological detail and immunohistochemical staining quality with all tested antibodies. DNA and RNA preservation, even after prolonged fixation time, was significantly better in RCLPE samples than in FFPE tissues.

**Conclusions:** In brain tumor samples, RCL2 fixation shows no significant compromise in terms of histology quality or immunoreactivity compared with formalin-fixed biopsies. However, nucleic acid quality is better preserved with RCL2 fixation.

**Reviewer's Comments:** Although this study examined brain tissue, the utility of RCL2 fixation has been reported in other tissue types. Given the general effectiveness and low cost of buffered formalin solutions, however, the use of other fixatives such as RCL2 will probably be limited to various research protocols or targeted ancillary molecular tests. (Reviewer-T. David Bourne, MD).

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Keywords: Tissue Fixation, Formalin, RCL2

Print Tag: Refer to original journal article
Flat epithelial atypia on a needle core biopsy with or without co-existing conventional atypical ductal hyperplasia can be associated with malignant outcome.

**Background:** Flat epithelial atypia (FEA), also known as columnar cell change with atypia, is described as an architecturally flat pattern of cells from one to several layers thick with low-grade cytologic atypia. The cells are characteristically columnar shaped with apical snouts and line a dilated lumen filled with secretions and microcalcifications. Since many mammographic biopsies are directed at microcalcifications, these lesions are not infrequently encountered. FEA is associated with atypical ductal hyperplasia (ADH), low-grade ductal carcinoma in situ (DCIS), and low-grade invasive carcinomas. The risk of a malignant outcome for FEA is clearly higher than with columnar cell change without atypia.

**Objective:** To determine the significance of FEA on preoperative needle core biopsy.

**Methods:** Archived breast biopsies were searched for various historical diagnostic terms related to columnar cell lesions (CCL), including columnar cell change/alterations, blunt duct adenosis, and/or ADH. Those confirmed to have a CCL without co-existing DCIS or invasive carcinoma were included, and findings were correlated to subsequent lumpectomy or mastectomy specimens.

**Results:** 4% of all biopsies had a CCL (256 of 6264), and 211 of these fit the study parameters of no co-existing DCIS or invasive carcinoma. Of these, 55% were CCL without atypia, 7% were FEA without co-existing ADH (15 of 211), and 19% were FEA with ADH (40 of 211). Ninety-four patients had a lumpectomy or mastectomy. DCIS and/or invasive carcinoma was found in 15% of patients with a CCL on preoperative biopsy (4 of 26), 37% of those with CCL and ADH (11 of 30), 14% of those with FEA alone (1 of 7), and 29% of those with FEA and ADH (9 of 31).

**Conclusions:** FEA frequently co-exists with typical ADH on needle core biopsy. Although FEA with ADH is more likely to have a malignant outcome than FEA alone, both may warrant conservative excision.

**Reviewer's Comments:** The findings seem to support the idea that a core biopsy with FEA, or columnar cell change with atypia, should be treated as ADH. (Reviewer-Mary T. Galgano, MD).

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Keywords: Flat Epithelial Atypia, Breast

Print Tag: Refer to original journal article
Pseudoinvasion in LH vs TAH

Evaluation of Vascular Space Involvement in Endometrial Adenocarcinomas: Laparoscopic vs Abdominal Hysterectomies.

Folkins AK, Nevadunsky NS, et al:

Mod Pathol 2010; 23 (August): 1073-1079

Pseudoinvasion is seen in both laparoscopic hysterectomy (LH) and total abdominal hysterectomy; however, LH is associated with more lymphovascular space invasion but more favorable features. The differentiation of pseudoinvasion versus true invasion may not be easily determined.

Background: Laparoscopic hysterectomy (LH) is increasing in popularity as an alternative to total abdominal hysterectomy (TAH), even for endometrial carcinoma cases. The changes in abdominal pressure and manipulation of the specimen with an intrauterine device have raised concern for an iatrogenic increase in positive washings by flushing tumor cells through the fallopian tubes. Surgeons now clamp the tubes or obtain washings before manipulating the intrauterine device to prevent this occurrence. Reports are now indicating an event considered “pseudovascular invasion” of endometrial carcinoma with postulation that this is due to the manipulator artifactually displacing tumor cells.

Objective: To compare true and artifactual lymphovascular space invasion (LVSI) of endometrial carcinomas in LH versus TAH.

Methods: 58 LH and 39 TAH cases with endometrial endometrioid adenocarcinoma grade 1 or 2 were reviewed for clinicopathologic features. Procedural artifacts were documented, including vertical endomyometrial clefts, inflammatory debris, benign endometrial glands, and disaggregated tumor cells in vascular spaces.

Results: Original pathology reports indicated LVSI in 16% of the LH and 7% of the TAH specimens. However, no feature (including pseudoinvasion) provided evidence that would have reliably differentiated LH from TAH on blinded review. Disaggregated intravascular cells were significantly associated with the reported presence of LVSI in both procedures, confirmed on review. Although the numbers were insufficient for statistical significance, LH had a higher index of LVSI but tended to have lower stage, less invasion, and fewer positive lymph nodes.

Conclusions: Vascular invasion tends to occur more frequently after LH than TAH, but this is associated with other favorable pathologic features. Pseudoinvasion occurs in both LH and TAH and is sometimes seen along with true LVSI. There is interobserver variability in the diagnosis of LVSI versus pseudoinvasion.

Reviewer’s Comments: The authors suggest that the presence of LVSI associated with less-aggressive pathologic features may indicate that, in some cases, pseudoinvasion and true invasion may be indistinguishable. (Reviewer-Mary T. Galgano, MD).

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Keywords: Laparoscopic Artifacts

Print Tag: Refer to original journal article
Antibodies Can Distinguish HCC From Other Lesions

Arginase-1: A New Immunohistochemical Marker of Hepatocytes and Hepatocellular Neoplasms.
Yan BC, Gong C, et al:

Arginase-1 appears to be a great marker of hepatocellular differentiation.

**Background:** Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world and the third most common cause of cancer death. Identifying the malignancy and distinguishing it from other malignancies are important for both prognosis and treatment. A number of antibodies are now available that can be used to help distinguish HCC from other malignancies. These include HepPar-1, CD10, and α-fetoprotein (AFP) as well as glypican-3, a marker of hepatocytic malignancy. These markers are not perfect, however, and HepPAR-1 (which correlates with an enzyme of the urea cycle) has been found to identify only approximately 50% of poorly differentiated HCCs in some studies.

**Objective:** To investigate the use of antibodies to arginase-1, another enzyme of the urea cycle, to distinguish HCC from other lesions.

**Methods:** Tissue microarrays as well as tissue blocks from 3 institutions were used for this study. Immunohistochemistry was performed with antibodies to arginase-1 and HepPAR-1. Staining was evaluated qualitatively and semi-quantitatively. Hep-PAR-1 and arginase-1 immunoreactivity were compared.

**Results:** Overall, the staining of 778 neoplasms (including 193 HCCs) was evaluated. HepPAR-1 antibodies stained 84% of all HCCs, whereas arginase-1 antibodies stained 96% of all HCCs. Of poorly differentiated HCCs, 46% were immunoreactive with antibodies to HepPAR-1, and 86% were immunoreactive with antibodies to arginase-1. Arginase-1 staining was both qualitatively and quantitatively superior in the staining of HCC. Arginase-1 immunoreactivity was seen in only 2 (a single intrahepatic cholangiocarcinoma and a chromophobe renal cell carcinoma) of 580 non-hepatocellular tumors tested, thus showing greater specificity than HepPAR-1 staining, present in 22 of the 580 cases (mostly gastric and colorectal adenocarcinomas). All benign hepatocytic lesions were strongly and diffusely immunoreactive with antibodies to arginase-1.

**Conclusions:** Arginase-1 is a sensitive and specific marker of hepatocellular differentiation. In this study, it functioned better than HepPAR-1.

**Reviewer's Comments:** Another weapon is now available for the pathologist needing to distinguish HCC from other tumors. If follow-up studies confirm these results, most pathologists will likely come to use arginase-1 as one of their primary antibodies to identify hepatocellular differentiation. (Reviewer-Edward B. Stelow, MD).
Deaths secondary to alcoholic liver disease have decreased over the past 20 years in the United States.

**Background:** Alcohol consumption continues to cause significant disease, and some estimate that it may account for up to 4% of global mortality. It is a significant factor in the development of liver disease (alcoholic liver disease) and accounts for 40% of deaths secondary to cirrhosis and 28% of all deaths due to liver disease. Alcohol consumption is an important contributing factor for the development of cirrhosis in patients with hepatitis C.

**Objective:** Because of the significant health implications and the need for public-based initiatives for the prevention of alcohol-related disease, this study investigated trends in alcoholic liver disease mortality in the United States over the past 20 years.

**Methods:** Mortality data were collected from a public data file that included death certificates for all 50 states. Both immediate and underlying causes of death were recorded, along with basic demographic data. Data were analyzed for trends over time from 1980 to 2003.

**Results:** Almost 250,000 deaths were attributed to alcoholic liver disease. Approximately 50,000 deaths were attributed to hepatitis C, and 3000 were attributed to hepatitis C and alcoholic liver disease. The median age of death was 55 years, and 71% of the decedents were men. Interestingly, the median age of death has fallen slightly over the years of the study, likely secondary to hepatitis C mortality. The percentage of alcohol-related liver disease deaths attributed to alcoholic hepatitis remained relatively constant over the years, at between 8.5% and 9.5%. The mortality rate for alcoholic liver disease decreased from 6.9 to 4.4 per 100,000 persons over the 20 years included in the study. In African Americans, the mortality rate has decreased from 11.6 to 4.1 over the 20 years.

**Conclusions:** Deaths due to alcoholic liver disease have decreased over the years but still are much more common than deaths due to hepatitis C.

**Reviewer’s Comments:** It is reassuring the see that alcoholic liver disease-related deaths have decreased and that there does not appear to be any upsurge in alcoholic hepatitis-related death secondary to binge drinking. One wonders about the truly significant decrease in deaths due to alcoholic liver disease for African Americans. (Reviewer—Edward B. Stelow, MD).

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Keywords: Cirrhosis, Hepatitis, Deaths, Alcohol

Print Tag: Refer to original journal article