**RET/PTC Rearrangement Not Only in Thyroid Cancers**

**RET/PTC Rearrangement Occurring in Primary Peritoneal Carcinoma.**


*Int J Surg Pathol;* 17 (June): 187-197

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**RET/PTC rearrangements occur in a small subset of nonthyroid papillary carcinomas.**

**Background:** The RET (rearranged during transfection) proto-oncogene codes for a tyrosine kinase receptor that plays an important role in the developmental regulation of the kidney and nervous systems. Sporadic mutations in RET have been well documented in cases of papillary thyroid carcinoma (PTC), the most common being RET/PTC 1 and RET/PTC 3. Although originally thought to be a specific tumor marker for PTC, RET/PTC rearrangements have also been observed in other thyroid tumors and non-neoplastic conditions, such as Hashimoto's thyroiditis and nodular hyperplasia.

**Objective:** To determine whether RET/PTC rearrangements are present in nonthyroid papillary tumors.

**Methods:** 57 nonthyroid tumors with a papillary growth pattern were retrieved from the pathology archives over a 16-year period at 2 hospitals in Ireland. Tumors included 15 primary peritoneal carcinomas, 10 papillary renal cell carcinomas, 10 ovarian serous carcinomas, 10 urothelial cell carcinomas, 5 endometrial serous carcinomas, 2 uterine endometrioid carcinomas, 1 uterine adenoacanthoma, and 4 mixed-phenotype carcinomas. Interphase fluorescence in situ hybridization (FISH) for RET/PTC rearrangement, interphase FISH for ploidy analysis, RT-PCR for RET/PTC 1 expression, immunohistochemistry (IHC) for RET oncoprotein expression, and BRAF mutation analysis were performed for each case. For FISH, the percentage of split RET signals was recorded. For reverse transcriptase polymerase chain reaction (RT-PCR), a score of negative (0) or positive (1) was assigned. For IHC, the intensity of immunoreactivity was classified as absent (0), weak (1), moderate (2), or strong (3). At least 10% of tumor cells had to show at least some degree of positivity for a case to be considered truly positive. Associations between RET/PTC rearrangement and various clinical and pathologic features were examined using Fisher's exact test, while FISH score comparisons were examined using a Mann-Whitney test (2-tailed; significance <0.05).

**Results:** Using FISH, approximately 9% of tumors had split RET signals above the cut-off level. These included 27% of primary peritoneal carcinomas and 10% of papillary renal cell carcinomas. Using RT-PCR, approximately 5% of tumors had detectable RET/PTC mRNA. All 3 cases were from the primary peritoneal carcinoma group. Using IHC, approximately 21% of tumors had detectable protein. These included 40% of primary peritoneal carcinomas, 20% of urothelial carcinomas, 18% of combined serous tumors, and 10% of papillary renal cell carcinomas. The BRAF T1799A mutation was not detected in any of the 57 tumors. Statistical analysis showed that the presence of an RET/PTC rearrangement was significantly associated with the primary peritoneal carcinoma tumor type.

**Conclusions:** RET/PTC rearrangements occur in a small subset of nonthyroid papillary carcinomas.

**Reviewer's Comments:** The authors advance the plausible suggestion that, based on the small percentage of positive cases, RET/PTC rearrangements in these nonthyroid papillary tumors may represent so-called secondary "passenger" type mutations, rather than mutational drivers directly influencing tumor growth.

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Are MECT1-MAML2 Gene Rearrangements Found in Bronchopulmonary MEC?

Mammalian Mastermind Like 2 11q21 Gene Rearrangement in Bronchopulmonary Mucoepidermoid Carcinoma.

De Oliveira Duarte Achcar O, Nikiforova MN, et al:

Hum Pathol; 40 (June): 854-860

MAML2 gene rearrangements can be detected by FISH or RT-PCR in the majority of bronchopulmonary mucoepidermoid carcinomas.

**Background:** Mucoepidermoid carcinoma (MEC) is one of the most common endobronchial malignancies of the respiratory tract. More than 75% of MECs of the salivary gland have been shown to have a recurrent chromosomal abnormality involving 2 genes: *mucoepidermoid carcinoma translocated 1* (*MECT1*) gene and *mammalian mastermind-like 2* (*MAML2*) genes. This results in the translocation t(11;19)(q21;p13). In the respiratory tract, adenosquamous carcinoma is frequently difficult to distinguish from MEC.

**Objective:** The authors performed a comparative analysis of MECT1-MAML2 gene rearrangements in bronchopulmonary MECs and other non-small-cell lung carcinomas.

**Methods:** 17 cases of bronchopulmonary MEC (10 low grade, 7 high grade), 16 adenosquamous lung carcinomas, 24 squamous cell lung carcinomas, and 41 lung adenocarcinomas were evaluated. Criteria used to distinguish high-grade MEC from adenosquamous carcinoma included exophytic endobronchial growth, surface epithelium lacking dysplasia, absence of individual cell keratinization, and the presence of a transition to low-grade MEC. Dual-color fluorescent in situ hybridization (FISH) was performed using the MAML2 break-apart probe on all cases. A positive result required >6.4% of nuclei showing a split signal. RNA was isolated from MEC and adenosquamous carcinoma cases, and reverse transcriptase polymerase chain reaction (RT-PCR) was performed for the MECT1-MAML2 gene fusion product.

**Results:** The MAML2 gene rearrangement was detected by FISH in 13 (77%) MECs: 3 (43%) high-grade cases and 10 (100%) low-grade cases. FISH was negative for rearrangement in all squamous cell carcinomas, adenosquamous carcinomas, and adenocarcinomas. The MECT1-MAML2 gene fusion was detected by RT-PCR in 6 (43%) of 14 MECs tested, in 2 (29%) of 7 high-grade cases, and in 4 (57%) of 7 low-grade cases. None of the adenosquamous carcinomas showed gene fusion products.

**Conclusions:** MAML2 gene rearrangements and MECT1-MAML2 gene fusion products can be detected by FISH and RT-PCR in a substantial number of bronchopulmonary MECs. This can be used to help distinguish high-grade MEC from primary lung adenosquamous carcinoma.

**Reviewer’s Comments:** This is the largest study to date examining the MECT1-MAML2 gene rearrangement in bronchopulmonary MEC. The MAML2 FISH detected gene translocation in all cases of low-grade bronchopulmonary MECs and in approximately 40% of high-grade MECs, similar to the results seen in salivary gland MECs. The lower percentage of detection in high-grade MECs may be due to other chromosomal abnormalities or misclassified adenosquamous carcinomas.

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Which Factors Predict MMR Protein Loss With Sebaceous Neoplasms?

Towards Identification of Hereditary DNA Mismatch Repair Deficiency: Sebaceous Neoplasm Warrants Routine Immunohistochemical Screening Regardless of Patient's Age or Other Clinical Characteristics.

Orta L, Klimstra DS, et al:
Am J Surg Pathol; 33 (June): 934-944

Mismatch repair protein abnormalities are common with sebaceous lesions.

Background: It has been recognized for >40 years that some patients are prone to develop visceral malignancies and sebaceous lesions of the skin. This variant of hereditary non-polyposis syndrome (HNPCC) is also termed Muir-Torre syndrome (MTS). A significant proportion of patients with HNPCC are found to have germline mutations of genes encoding the mismatch repair (MMR) proteins. As such, immunohistochemistry for these proteins has been shown to be helpful for identifying patients with colon cancers who are at risk for having HNPCC. Few studies have been performed, however, that have shown the use of immunohistochemistry with sebaceous lesions for the identification of patients with HNPCC or MTS.

Objective: To investigate the use of immunohistochemistry for MMR proteins with sebaceous lesions and the clinicopathologic features associated with protein loss.

Methods: All cases diagnosed as sebaceous hyperplasia, adenoma, and carcinoma were identified within a single institution's surgical pathology database. Cases with available material for immunohistochemistry were used. Clinical and family histories were gathered. Immunohistochemistry was performed with antibodies to MLH1, MSH2, MSH6, and PMS2. Tumor infiltrating lymphocytes and peritumoral lymphocytic response were assessed using immunohistochemical staining with antibodies to CD3.

Results: 29 patients were identified who had one or more sebaceous lesions. Two patients had sebaceous hyperplasia only and had intact MMR proteins. In the other 27 patients, a total of 36 sebaceous carcinomas, 14 sebaceous adenomas, and 7 sebaceous hyperplasias were identified. Loss of MMR proteins by immunohistochemistry was identified in 12 patients. Concurrent loss of MSH2 and MSH6 was seen in 8 patients, concurrent loss of MLH1 and PMS2 was seen in 2 patients, and loss of only MSH2 or MSH6 was seen in 1 patient, each. In any given patient, all sebaceous neoplasms showed identical staining patterns. Patients with noncutaneous tumors also had identical staining patterns with their noncutaneous tumors. Patients with abnormal MMR protein immunohistochemistry were younger, were more likely to have tumors of the trunk or extremities, and were more likely to be male. They were also more likely to have visceral malignancies. All patients who fulfilled the Amsterdam or Bethesda criteria for HNPCC had abnormal MMR protein immunohistochemistry. Tumors with abnormal immunohistochemistry had more tumor-infiltrating lymphocytes and a more prominent peritumoral lymphocytic response.

Conclusions: Although a number of factors help predict MMR protein loss with sebaceous neoplasms, the authors suggest that all lesions be immunostained if one wishes to identify patients with HNPCC. This is because the lesions are rare, and no clinical or histologic factors can be used that entirely exclude the possibility of disease.

Reviewer's Comments: This is a relatively large study investigating the use of MMR protein immunohistochemistry with sebaceous lesions. Nearly half the patients with these lesions show abnormalities of MMR protein expression.

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CCL Associated With Breast Calcifications on Vacuum-Assisted Biopsy

Columnar Cell Lesions Associated With Breast Calcifications on Vacuum-Assisted Core Biopsies: Clinical, Radiographic, and Histological Correlations.
Senetta R, Campanino PP, et al: Mod Pathol; 22 (June): 762-769

Columnar cell lesions with luminal microcalcifications are commonly seen in biopsies performed for a BI-RADS3 category. A more worrisome pattern of calcifications may be associated with an adjacent lesion.

**Background:** Breast calcifications detected by mammography are a frequent indication for core biopsies, and the Breast Imaging Reporting and Data System (BI-RADS) has standardized these findings for clinicopathological correlation. Given the propensity for microcalcifications in the flocculent secretions of columnar cell lesions (CCL), these are not an infrequent finding in vacuum-assisted core biopsy.

**Objective:** To specifically correlate CCL with radiographic interpretation and clinical outcome.

**Participants/Methods:** 392 women underwent vacuum-assisted stereotactic biopsy performed for histological characterization of the sole radiographic feature of microcalcifications (excluding those with architectural distortion, structural thickening, opacities, etc). Of these biopsies, 156 were diagnosed along the spectrum of CCL, and 137 (88%) of these had calcifications within the CCL and were included in the study. The remaining 236 biopsies were not associated with a CCL and were excluded from further review. Two mammographers reviewed each case for a consensus on a BI-RADS classification. Two breast pathologists reviewed each case and diagnosed the CCL as either CCL without atypia or flat epithelial atypia (FEA). The features required for FEA included a flat proliferation of atypical cells within the terminal duct lobular unit having a loss of polarity or stratified nuclei. Complex architecture (including cribriform spaces) and micropapillae were classified as either atypical ductal hyperplasia (ADH) or low-grade ductal carcinoma in situ (DCIS). Benign lesions without calcifications were considered inadequate B1; CCL without atypia was classified as B2; FEA, ADH, papillomas, and/or LCIS were considered B3; FEA involving 4+ cores was classified as B4; and CCL associated with DCIS or invasive carcinoma was considered B5a or B5b.

**Results:** Vacuum-assisted biopsies obtained for microcalcifications determined to be associated with CCL represented 37% of all breast biopsies and 62% of those in the BI-RADS3 category. None of the BI-RADS5 calcifications were accounted for by a CCL alone. The women with CCL had comparable age and menopausal status as those with a non-CCL, but atypia was associated with long-term hormonal therapy in both groups. Biopsies with no worse findings than FEA were not associated with a malignancy after surgical excision of the lesion.

**Conclusions:** In cases of low-risk microcalcifications (BI-RADS3) determined to be within a CCL on core biopsy, a worse lesion is not commonly found on surgical follow-up. However, more worrisome microcalcifications can still be seen in ADH or DCIS that is adjacent to a CCL, and these should continue to be treated with surgical excision.

**Reviewer’s Comments:** The methods of this report excluded cases in which the calcifications were associated with lesions other than the CCL, which would include adjacent DCIS with microcalcifications.

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CXCR7 Expression Predicts DFS in Stage I NSCLC

Higher Expression of Chemokine Receptor CXCR7 Is Linked to Early and Metastatic Recurrence in Pathological Stage I Nonsmall Cell Lung Cancer.

Iwakiri S, Mino N, et al:
Cancer; 115 (June 1): 2580-2593

High CXCR7 expression in stage I non-small-cell carcinomas is associated with a decreased 5-year disease-free survival.

**Background:** Lung carcinoma is the leading cause of cancer-related death in developed countries. Chemokine receptors have been demonstrated to play a role in cancer metastasis in several organ systems, including breast carcinoma, melanoma, and gastric carcinoma.

**Objective:** To analyze the expression of all known chemokine receptors in lung cancer samples, and to correlate the chemokine receptor levels with EGFR mutation status and clinical outcome.

**Methods:** 11 lung carcinoma cell lines and 127 consecutive samples from patients with non-small-cell lung cancer (NSCLC) who underwent resection were included. All patient samples were immediately snap-frozen after resection. Total RNA was isolated from cell line samples, and reverse transcriptase polymerase chain reaction (PCR) was performed for quantitative amplification of all known chemokine receptor mRNAs (CCR1-11, CXCR1-7, XCR1, and CX3CR1). The chemokine receptors that showed increased expression in cell lines were similarly investigated in patient samples. Genomic DNA was extracted from each sample, and all EGFR exons were amplified by PCR. Single-strand conformational polymorphism PCR was used to detect mutations; if present, the corresponding sample was sequenced to determine the mutation type.

**Results:** All 11 cell lines showed substantial expression of chemokine receptors CXCR3 and CXCR4. CXCR7 showed higher expression in squamous cell carcinoma than adenocarcinomas and was not observed in small-cell carcinoma or large-cell carcinoma lines. In patient samples, there was no significant difference in expression of CXCR3 when stratified by patient demographics or outcome. For pathologic stage I NSCLCs, CXCR4 and CXCR7 were significantly higher in patients with postoperative recurrent/metastatic disease than in those without recurrent/metastatic disease. The 5-year disease-free survival (DFS) was 63% for patients with high CXCR7 expression versus 85% for patients with low CXCR7 expression ($P = 0.03$). However, the difference in 5-year overall survival (OS) was not significant. Patients with stage II or higher disease did not show a significant difference in DFS or OS when stratified by CXCR7 expression.

**Conclusions:** Higher expression of CXCR7 is associated with postoperative recurrence/metastasis and worse 5-year DFS in patients with stage I NSCLC but does not appear to affect overall survival.

**Reviewer's Comments:** High CXCR7 expression in stage I NSCLC is associated with a low 5-year DFS. This may allow selection of a subset of patients with stage I disease to receive adjuvant therapy immediately after surgery. In addition, chemokine receptors are a potential target for molecular-based targeted therapy in the future.

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Will Anti-EGFR Drugs Work in KRAS Mutated Colorectal Cancer?

Clinical Relevance of EGFR- and KRAS-Status in Colorectal Cancer Patients Treated With Monoclonal Antibodies Directed Against the EGFR.


KRAS mutations appear to activate a carcinogenic pathway downstream and confer tumor resistance to the anti-EGFR mAb agents cetuximab and panitumumab, which should be administered only to patients with wild-type KRAS colorectal cancers.

**Objective:** To review molecular mechanisms of epidermal growth factor receptor (EGFR) interactions with the Ras/MAPK pathway and the clinical relevance of KRAS mutations on anti-EGFR directed therapies in colorectal cancer.

**Discussion:** EGFR (HER1) belongs to the erbB receptor tyrosine kinase family, along with erbB2 (HER2/neu), erbB3 (HER3), and erbB4 (HER4). Binding of endogenous ligands (epidermal growth factor, transforming growth factor-alpha, among others) to the EGFR extracellular domain causes either EGFR - EGFR homodimerization or EGFR heterodimerization with other erbB receptors leading to autophosphorylation of the intracellular EGFR tyrosine kinase domain, which activates a number of intracellular signaling pathways, most notably (1) RAS-RAF-MEK-MAPK control of gene transcription, cell cycle, etc. and (2) the anti-apoptotic PI3K-Akt pathway. Immunohistochemical detection of EGFR in colorectal cancer is common, but the extent of staining does not correlate well with EGFR gene amplification or predict response to anti-EGFR monoclonal antibody (mAb) therapy (cetuximab or panitumumab); eg, 25% of EGFR negative tumors still respond to cetuximab. Increased EGFR gene copy number can be demonstrated by in situ hybridization or polymerase chain reaction (PCR), methods that do not distinguish gene amplification from polysomy and do not necessarily correlate with each other. In most studies, increased EGFR gene copy number predicts the response to mAb therapy. In colorectal cancer, EGFR mutations are uncommon and do not predict the level of response to EGFR-directed therapy. KRAS mutations are demonstrated in 16% to 38% of non-small-cell lung cancers, roughly 40% of colorectal cancers, and >90% of pancreatic cancers. Roughly 90% activating KRAS mutations occur in codons 12 and 13. These are detected by direct sequencing (dideoxy- or pyrosequencing methods), allele-specific PCR, and allele-specific PCR/melting curve analysis. Numerous studies show that the benefit from anti-EGFR mAb therapy, either alone or as a combined regimen, is restricted to wild-type KRAS tumors and is absent from colorectal tumors with KRAS mutation. So far, small molecule EGFR tyrosine kinase inhibitor therapies have no proven benefit in colorectal cancer, in contrast to lung cancer.

**Conclusions:** KRAS mutations appear to activate a carcinogenic pathway “downstream” and confer tumor resistance to the anti-EGFR mAb agents cetuximab and panitumumab, which should be administered only to patients with wild-type KRAS colorectal cancers.

**Reviewer’s Comments:** The anti-EGFR mAb agents cetuximab and panitumumab are approved for use in refractory colorectal cancers and advanced head and neck squamous cell carcinomas (cetuximab). The small molecule EGFR tyrosine kinase inhibitors erlotinib and gefitinib are approved for use in platinum-refractory non-small-cell lung cancers. In lung cancer, increased EGFR copy number predicts response to erlotinib and gefitinib, as does Asian race, female sex, absence of smoking history, and adenocarcinoma histology. In non-small-cell lung cancer, KRAS mutations predict non-response to EGF tyrosine kinase inhibitors.

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Hurry Up and Wait—Improving Test Result Turnaround Times

Analysis of Turnaround Time by Subdividing Three Phases for Outpatient Chemistry Specimens.

Chung H-J, Lee W, et al:
Ann Clin Lab Sci; 39 (2): 144-149

Time spent by patients waiting for phlebotomy may significantly contribute to delayed test result turnaround times.

**Background:** The definition of test result turnaround time (TAT) depends on a number of factors, including physician expectations, specimen type, and the analyte being measured. TAT has traditionally been divided into preanalytical, analytical, and postanalytical phases of testing.

**Objective:** To report the results of a laboratory information system (LIS)-based monitoring program that automatically calculates, records, and analyzes time to complete 3 predefined phases of chemistry testing.

**Methods:** For study purposes, TAT was defined as time from printing the initial specimen barcode to reporting the result to the hospital information system (HIS). A total of 13,594 specimens were collected (207,143 total tests performed), for which various routine chemistry analytes were assessed (total plasma calcium, glucose, creatinine, etc). The LIS automatically recorded 4 separate time points: time of initial barcode label printing, scanning of the barcode by the autoanalyzer, result reporting to the LIS, and result reporting to the HIS. The preanalytical phase began when the specimen barcode was first printed and included simultaneous specimen accessioning, waiting for phlebotomy, phlebotomy, specimen transport to the laboratory, manual centrifugation, and manual loading of the specimen into the analyzer. The analytical phase began when the specimen barcode was scanned by the autoanalyzer, followed by order retrieval from the LIS, analysis, and result transfer to the LIS. The postanalytical phase began when the result was received by the LIS and ended with manual or automatic verification and result reporting to the HIS. For all 13,594 specimens, the mean TAT and standard deviation were reported. The average time taken in each phase was recorded, as was the contribution of each phase to the overall TAT. TATs were divided into 3 groups: within 60 minutes, between 60 and 90 minutes, and >90 minutes.

**Results:** The average TAT was 43.6 7.7 minutes. Completion times for each of the testing phases were 29.7 6.9 minutes (preanalytical), 13.9 4.1 minutes (analytical), and 0.02 0.13 minutes (postanalytical); 98% of the specimen results were reported within 60 minutes. Preanalytical phase TAT delays were responsible for the majority of results reported between 60 and 90 minutes, while analytical phase delays were responsible for the majority of results reported after 90 minutes. The cause of most delays within the preanalytical phase was the "waiting for phlebotomy" step.

**Conclusions:** LIS-based TAT measurements provide accurate and useful information regarding test result reporting. Most routine chemistry results are reported in <60 minutes, and the cause of most delays is patient time spent waiting for phlebotomy.

**Reviewer's Comments:** The authors provide important insights into an effective TAT tracking strategy utilizing LIS. In labs with high-specimen volume, such data gathering helps define areas needing technical or personnel improvements.

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Cytoscrape--Alternative to Immunohistochemistry on FNA

A technique to Improve Diagnostic Information From Fine-Needle Aspirations: Immunohistochemistry on Cytoscrape.

Skov BG, Kiss K, et al:
Cancer Cytopathol; 117 (April 25): 120-127

The cytoscrape technique is feasible and yields malignant cells in >90% of cases. TTF1 staining could be performed reliably with this material.

Background: Fine-needle aspiration (FNA) is frequently used in the diagnosis of pulmonary lesions. While cytology is good at distinguishing small-cell carcinoma from non-small-cell carcinoma, determining subtypes such as adenocarcinoma versus squamous cell carcinoma can be difficult. New treatment modalities require differentiation between these subtypes, due to efficacy and mortality data. Thyroid transcription factor-1 (TTF1) is positive in the majority of lung adenocarcinomas but not in most lung squamous cell carcinomas.

Objective: To describe a cytoscrape technique for immunohistochemical staining in pulmonary FNAs.

Participants/Methods: 47 patients with lung neoplasms who underwent FNA were included. All smears were air-dried and stained with Diff-Quick or May-Grunwald-Giemsa. After smears were read, the neoplastic cells were gently scraped off with a surgical blade without destaining. These cells were then placed into a tissue cassette covered by Millipore paper, and a clot was formed with 3 drops of human plasma and 1 drop of human thrombin. The clots were processed as routine paraffin-embedded material, and sections were cut for hematoxylin and eosin, mucin, and TTF1. A cut-off of 10% of cells staining was considered positive for TTF1 evaluation. TTF1 and mucin staining on histological resection specimens was considered the gold standard (available in 21 cases). Reference specimens (made from touch preps of thyroid neoplasms, colorectal carcinomas, and other tumors to ensure accuracy of cytoscrape immunohistochemistry) were also stained with TTF1 and mucin.

Results: All cytoscrape preparations made from reference materials stained appropriately; the sensitivity and specificity of TTF1 staining for thyroid tissue on cytoscrape was 100%, and mucin was identified in 60% of colorectal carcinomas. On FNA samples, 43 (91%) cytoscrapes contained at least 50 malignant cells. The cellular morphology on cytoscrape was inferior to that on smear preparations. TTF1 staining was positive in 14 (33%) of 42 cytoscaped tumors; the sensitivity and specificity of TTF1 staining on cytoscrape was 50% and 100%, respectively. Mucin staining was present in 9 (21%) of 42 cytoscaped tumors; the sensitivity and specificity of mucin staining on cytoscrape was 71% and 58%, respectively. In 31 (72%) cases, the cytologic diagnosis was more specific with the addition of staining results.

Conclusions: The cytoscrape technique was feasible and yielded malignant cells in >90% of cases. Immunohistochemistry with TTF1 can be useful in distinguishing primary lung adenocarcinomas from other tumors when used with the cytoscrape technique. However, a negative TTF1 result does not rule out adenocarcinoma in this setting.

Reviewer's Comments: The described technique offers an alternative method of immunohistochemical staining when conventional cell block preparations are not available. However, in samples with scant malignant cells, this method may also have a lower diagnostic yield.

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New Prognostic System for Primary Myelofibrosis More Discriminatory

New Prognostic Scoring System for Primary Myelofibrosis Based on a Study of the International Working Group for Myelofibrosis Research and Treatment.

Cervantes F, Dupriez B, et al:

Blood; 113 (March 26): 2895-2901

A prognostic scoring system for primary myelofibrosis based on 5 presenting features (Hb, WBC, circulating blasts >1%, age, and constitutional symptoms) effectively stratifies patients into 4 distinct survival groups.

**Objective:** To establish a prognostic system for primary myelofibrosis that identifies patients who are candidates for new and investigational therapies.

**Background:** Established therapies for primary myelofibrosis are lacking, so investigators are looking toward allogeneic stem cell transplantation and experimental small molecule drugs directed against JAK2 (eg, oral JAK2 inhibitor INCBO18424, the multikinase inhibitor MK-0457).

**Methods:** 1054 patients at 7 institutions were diagnosed between January 1980 and April 2007 with primary myelofibrosis. They were conservatively managed and/or treated with single-agent hydroxyurea, busulfan, 6-mercaptopurine, androgens, erythropoietin, steroids, interferon, anagrelide, thalidomide, lenalidomide, cladribine, splenectomy (n=111), or bone marrow transplant (n=7). Survival from diagnosis was calculated by the Kaplan-Meier method.

**Results:** Only 5% of the cohort was <40 years old. The median survival was 69 months (95% CI, 61 to 76 months). Thirty percent of patients had cytogenetic abnormalities, and 59% of those tested had JAK2 V617F mutations. By multivariate analysis, presenting features including hemoglobin (Hb) <10 g/dL, WBC >25,000, circulating blasts >1%, age >65 years, and constitutional symptoms predicted unfavorable survival. Hb <10 g/dL was the strongest prognostic indicator. Four prognostic groups (high risk, 3 factors; intermediate risk-2, 2 factors; intermediate risk-1, 1 factor; low risk, no factors) stratified patients into statistically significant survival groups, with a median survival of 27, 48, 95, and 135 months, respectively (P <0.001). Thrombocytopenia was not prognostically significant when Hb was >10 g/dL. Monocytosis and splenomegaly at diagnosis were not statistically significant. Cytogenetic abnormalities were associated with shorter survival but also with Hb <10 g/dL. JAK2 status was not prognostically significant.

**Conclusions:** A prognostic scoring system for primary myelofibrosis based on 5 presenting features (Hb <10 g/dL, WBC >25,000, circulating blasts >1%, age >65 years, and constitutional symptoms) effectively stratifies patients into 4 distinct survival groups.

**Reviewer’s Comments:** According to the authors, this new prognostic system for primary myelofibrosis is more discriminatory than previously proposed systems. As new therapies emerge, primary myelofibrosis patients with low-risk presenting features and expected median survival >11 years may not be candidates for bone marrow transplant or investigational drugs, but high-risk patients with an expected median survival of 2 years may be.

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UC Plus PSC Increases Risk of Colonic Dysplasia

*Pathologic Features of Ulcerative Colitis in Patients With Primary Sclerosing Cholangitis: A Case-Control Study.*
*Am J Surg Pathol; 33 (June): 854-862*

Some histologic features are more common in patients who have PSC and UC than in those with UC only.

**Background:** It has been recognized for nearly 50 years that ulcerative colitis (UC) is frequently associated with primary sclerosing cholangitis (PSC). Approximately 5% of patients with UC are found to also have PSC, whereas 23% to 80% of patients with PSC are noted to have UC. Recently, some studies have suggested that PSC associated with UC is unique from UC only. Other studies have recently reported specific histologic features in UC associated with PSC, as well as other prognostic differences for the disease, compared to UC only.

**Objective:** To investigate the pathologic features of UC seen in patients with and without concomitant PSC.

**Methods:** The study consisted of 40 patients with UC alone and 40 matched patients with UC associated with PSC. Materials from 3 academic institutions were used. Clinical and follow-up information was gathered. In total, 872 biopsy specimens and 30 colectomy specimens were reviewed. Chronic changes and activity were assessed in all specimens semi-quantitatively. The presence or absence of pancolitis, subtotal colitis, rectal sparing, and patchy disease was noted. Biopsy findings from terminal ilium specimens were also noted.

**Results:** Patients with UC associated with PSC presented at a slightly younger age than patients with UC alone. Of the patients with UC and PSC, a slight majority had well-established UC before their diagnosis of PSC, and 11 patients underwent liver transplantation during their disease course. No significant differences were found between groups in the number of biopsies or specimen types, or in the overall treatments. Patients with UC associated with PSC were more likely to have pancolitis, although they had lower overall grades of their disease. They also more often had cecal activity. The presence of ileitis was similar between groups. There was no statistical difference in the number of patients who developed pouchitis, although it was nearly 60% more common in those with PSC. Although a statistical difference was not seen between groups as far as the development of dysplasia was concerned, patients with UC and PSC were twice as likely to develop dysplasia and were the only patients to have high-grade dysplasia. No adenocarcinomas developed in either patient group.

**Conclusions:** Patients with UC associated with PSC are more likely to have pancolitis with less severe disease. Other findings are generally similar compared to those of patients with UC without PSC.

**Reviewer's Comments:** This interesting study seems slightly limited by the number of cases. It is interesting to note the trend toward more and higher-grade colonic dysplasia in patients with UC and concomitant PSC.

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Are Mucinous Ovarian Tumors Chemoresistant?

Clinicopathological Characteristics of Mucinous Adenocarcinoma of the Ovary.

Shimada M, Kigawa J, et al: Gynecol Oncol; 113 (June): 331-334

Invasive mucinous adenocarcinomas of the ovary are chemoresistant, but they have similar outcome data as stage-matched serous carcinomas as long as they can be optimally debulked.

**Background:** Mucinous ovarian tumors represent approximately 10% of ovarian carcinomas, but difficulty in accurate classification and histologic interpretation of invasion complicates evaluation of their clinicopathologic features. However, mucinous carcinomas are thought to be relatively chemoresistant with a worse prognosis, similar to ovarian clear cell carcinomas. Given that the current standard of care for ovarian carcinoma includes cytoreductive surgery with postoperative chemotherapy, the accurate description of chemoresponse in mucinous carcinomas could have therapeutic implications.

**Objective:** To centrally review and classify mucinous tumors of the ovary and to collect clinicopathologic data.

**Participants/Methods:** Of 1400 patients diagnosed with ovarian carcinoma, 189 were classified as having mucinous adenocarcinoma and consented to have their pathologic specimens available for centralized review. All patients underwent primary cytoreductive surgery and platinum-based chemotherapy. Tumors were reviewed and, in brief, were considered intraepithelial carcinoma if there was no invasion but there was severe cytologic atypia, regardless of stratification or complex intracystic growth. Microinvasion was defined as invasive foci up to 5 mm in linear dimension in any focus. Invasion beyond 5 mm in any linear dimension was considered invasive adenocarcinoma; 433 patients with serous ovarian carcinoma served as a control group.

**Results:** 25 cases were reclassified as endometrioid (8), clear cell (5), serous (3), or mixed (4) type. Thirteen cases were reclassified as metastatic carcinoma, including 7 pseudomyxoma peritonei; 64 cases were confirmed as mucinous invasive adenocarcinoma, 45 as mucinous intraepithelial carcinoma, and 42 as mucinous tumors of borderline malignancy. Of invasive mucinous carcinomas, 45 were International Federation of Gynecology and Obstetrics stage I to II and 19 were stage III to IV. In cases with optimal cytoreduction, there was no survival difference between stage-matched mucinous and serous carcinomas. However, in those with suboptimal cytoreduction, mucinous adenocarcinomas had a significantly worse survival than did serous carcinomas (27.8% vs 61.5%). Mucinous tumors were noted to have a significantly lower response rate to chemotherapy than did serous tumors (12.5% vs 67.7%).

**Conclusions:** When carefully classified, invasive mucinous adenocarcinomas of the ovary have a similar prognosis to that of stage-matched serous carcinomas, only if optimally debulked. Mucinous adenocarcinomas that were sub-optimally debulked had a poor response to chemotherapy and worse prognosis than did serous carcinomas.

**Reviewer's Comments:** This study is interesting in that it highlights the difficulties of classifying mucinous tumors of the ovary that depend heavily on (1) grossing techniques to thoroughly sample these highly variable lesions and (2) histologic interpretation of cytology and stromal invasion. But for surgeons, this manuscript highlights the importance of optimal cytoreduction in mucinous adenocarcinomas because of a poor response to current chemotherapy regimens.

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Multilayered Epithelium Possible Marker of GERD

Multilayered Epithelium in Mucosal Biopsy Specimens From the Gastroesophageal Junction Region Is a Histologic Marker of Gastroesophageal Reflux Disease.


Multilayered epithelium may be a precursor lesion of intestinal metaplasia of the esophagus.

**Background:** Barrett esophagus (BE) and gastroesophageal reflux disorder (GERD) are considered risk factors for the development of esophageal adenocarcinoma. Intestinal metaplasia with goblet cells of the esophageal squamous mucosa is considered necessary for a diagnosis of BE. Other possible precursor or intermediate lesions include cardiac-type mucosa, cardiac-type mucosa with goblet cells, and "multilayered epithelium" (ME). ME of the esophagus is somewhat akin to early squamous metaplasia of the cervix with apparent glandular epithelium overlying apparent squamous epithelium. It has been described in patients with BE in both retrospective and prospective studies.

**Objective:** To evaluate the presence of ME in patients with GERD, and to compare its phenotype to known and possible precursor lesions of esophageal adenocarcinoma.

**Methods:** Gastroesophageal junction biopsies were obtained from patients with and without symptoms of GERD. These included 27 patients with GERD and BE, 13 patients with GERD but without BE, and 18 patients without GERD or BE. Samples were reviewed for the presence of ME. Glandular epithelium was categorized as goblet-cell type, mucinous, oxyntic, or combined mucinous and oxyntic. Proximal biopsies were evaluated for histologic features of esophagitis. Immunohistochemistry was performed with antibodies to MUC2, MUC5AC, MUC6, CDX2, p63, and CK13.

**Results:** ME was present in 33% of patients with BE and GERD without BE. No patients without GERD had ME. ME was always found at the surface and extended into deeper glands about half the time. It was found adjacent to goblet cells in about half the patients with BE. Goblet cells were present in all BE cases, in 8% of patients with GERD without BE, and in 28% of patients without GERD or BE. ME was immunoreactive with antibodies to MUC2 in 38% of cases and to CDX2 in 77% of cases. Interestingly, p63 positivity was also seen in only 38% of cases. MUC2 and CDX2 immunostaining were seen in 0% and 24% of cases of columnar epithelium without goblet cells, respectively. Both were present in all cases of BE.

**Conclusions:** ME can certainly be used as a marker for GERD. It may also represent an intermediate lesion of metaplasia, between columnar epithelium without goblet cells and that with goblet cells (BE).

**Reviewer's Comments:** This is an interesting study that again demonstrates our relatively poor understanding of the development of BE in patients with GERD. The relationship of ME to gastric cardiac mucosa remains to be elucidated.

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Can Flow Cytometry Help Evaluate Hodgkin Lymphoma?

Flow Cytometry Can Diagnose Classical Hodgkin Lymphoma in Lymph Nodes With High Sensitivity and Specificity.
Fromm JR, Thomas A, Wood BL:
Am J Clin Pathol; 131 (March): 322-332

Using 9-color flow cytometry, neoplastic Hodgkin cells demonstrate CD30, CD40, and CD95 staining, increased forward and side scatter, weak CD45, absence of CD64, and absent to dim CD20 staining.

Background: Flow cytometry has not been applied to the diagnosis of classic Hodgkin lymphoma due to the relative paucity of malignant cells and the overwhelming predominance of background reactive lymphocytes.

Objective: To characterize a flow cytometric 9-color assay for evaluation of classic Hodgkin lymphoma.

Methods: 317 random lymph node specimens and 141 selected specimens with clinical or morphologic suspicion of classic Hodgkin lymphoma were evaluated by the following single-tube, 9-color flow cytometry assay at the University of Washington between July 2005 and June 2006. Of 317 random cases, 38 were excluded due to having <50,000 viable events. Cell suspensions were incubated in a single tube with 9 antibodies (CD95-PB, CD64-FITC, CD30-PE, CD45-ECD, CD40-PE-Cy5.5, CD20-PE-Cy7, CD15-APC, CD71-APC-A700, and CD5-APC-Cy7) and were analyzed on a modified 4-laser, 10-color Becton Dickinson LSRII flow cytometer.

Results: Neoplastic Hodgkin and Reed Sternberg (HRS) cells were defined by positive CD30, CD40, and CD95 staining, increased forward and side scatter, weak CD45, absence of CD64, and absent to dim CD20 staining. When present, subpopulations of HRS-cell-T-cell rosettes demonstrated a combined immunophenotype (CD5+, CD15+, CD20-, CD30+, bright CD40+, CD64-, CD71+, and bright CD95+) with bright CD45. Among 279 unselected cases, there were no false-positive flow cytometric diagnoses (100% specificity), and 8 of 10 classic Hodgkin lymphomas were identified. Among 141 selected cases, 39 of 43 (91%) classic Hodgkin lymphomas were correctly identified with no false-positive results. Overall, the single-tube, 9-color flow cytometry assay was 88.7% sensitive and 100.0% specific.

Conclusions: A carefully constructed, 9-color flow cytometry assay can accurately identify classic Hodgkin lymphoma.

Reviewer's Comments: The authors are working on 5- and 6-color combinations to achieve the same end. Readers should note that weak CD95 and CD15 staining can be seen in small-cell undifferentiated carcinoma too. A subset of Hodgkin cells is bound by T-cell rosettes, which contribute a chimeric T-cell phenotype (CD3, CD5, and bright CD45) to tumor cells.

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Does Colonoscopy Still Reign for Detecting Colorectal Cancer?

A Simplified, Noninvasive Stool DNA Test for Colorectal Cancer Detection.

Itzkowitz S, Brand R, et al:
Am J Gastroenterol; 103 (November): 2862-2870

A second-generation stool DNA test using DNA-stabilizing buffer and testing for hypermethylation of the vimentin gene is 83% sensitive and 82% specific for colorectal cancer.

Background: A first-generation stool DNA assay demonstrated superior sensitivity and comparable specificity to the Hemoccult II fecal occult blood test. However, DNA degradation during specimen transport was a major limitation to the prototype assay. A second-generation assay using DNA-stabilizing buffer at collection and testing for hypermethylation of the vimentin gene has been developed.

Objective: To evaluate a second-generation stool DNA test as a screening assay for colorectal cancer.

Methods: Stool was collected in a DNA-stabilizing buffer and sent by mail to the testing laboratory. DNA was extracted as described for the first-generation assay. Multiplex real-time polymerase chain reaction (PCR) for 4 different-sized DNA fragments at each of 2 loci (5p21 [Locus D] and LOC91199 [Locus Y]) was used to confirm DNA integrity (the DIA-DY test). Methylation-specific PCR was applied to determine hypermethylation of the vimentin gene.

Results: In a validation set consisting of 42 colorectal cancer patients and 241 with normal colonoscopy, vimentin hypermethylation was 81% sensitive in detecting colorectal cancer. The DY assay was 60% sensitive. Combined, the 2 tests were 86% sensitive, with one of the tests detecting colorectal cancer in 36 of 42 patients. Vimentin hypermethylation and the DY assay were 82% and 85% specific, respectively.

Conclusions: A second-generation stool DNA test using DNA-stabilizing buffer and testing for hypermethylation of the vimentin gene is roughly 83% sensitive and 82% specific for colorectal cancer.

Reviewer's Comments: Colonoscopy is still the optimal colorectal cancer screening method. However, population compliance and adequacy of bowel preparation remain as limiting factors for colonoscopy. Therefore, a non-invasive screening test that does not require bowel preparation has the potential to save many lives.

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Micro-RNA Profiling Useful for FFPE Tissues


Global micro-RNA profiling, unlike more traditional RNA analyses, may be successfully conducted using archival formalin-fixed paraffin-embedded tissues.

Background: Micro-RNAs (miR) are short non-coding RNAs first discovered in 1993 in the nematode Caenorhabditis elegans. It is estimated that miRs may represent up to 4% of protein-coding genes, making it one of the largest classes of regulatory genes. Unlike mRNA, miRs exhibit imperfect complementarity, meaning that a single miR may bind to hundreds of target genes. This property explains why miRs are involved in functions that range from regulation of transporters to transcription factors. In addition, miRs show much greater resistance to deleterious effects of formalin fixation and paraffin embedding.

Objective: To demonstrate the validity and utility of a high-throughput platform for miR quantitation using formalin-fixed paraffin-embedded (FFPE) tissue.

Methods: The authors performed quantitative real-time polymerase chain reaction (PCR) miR expression profiling using 34 FFPE breast lumpectomy specimens containing invasive ductal adenocarcinoma and 6 FFPE reduction mammoplasty specimens with no evidence of malignancy. The expression level of a total of 365 miRs was determined. After RNA purification from FFPE samples, reverse transcriptase PCR analysis was performed, followed by global miR profiling using the Taqman Low Density Array (TLDA). The correlation between paired frozen and FFPE breast cancer samples was determined. Technical reproducibility between TLDA and PCR results was also determined, followed by PCR validation and comparison of miR profiles of breast tumor and normal breast tissues.

Results: Expression profiles showed a high correlation between all technical replicates (Spearman's rho was above 0.90 in all cases), supporting that TLDA profiles of miR expression in FFPE samples are highly reproducible. There was also a high correlation between miR profiles of FFPE and frozen tumor samples obtained from the same tumor (Spearman's rho of at least 0.94). Compared to normal breast tissue, FFPE breast cancer samples showed a panel of miRs that were consistently dysregulated. These included the previously described upregulated miR-21, miR-155, miR-191, and miR-196a, as well as the downregulated miR-125b and miR-221. Novel miRs were also discovered.

Conclusions: Global miR profiling of human breast cancers is a technically valid and useful method using FFPE tissue. Such a method has potentially useful application in the evaluation of archival banks of other types of FFPE human tissues.

Reviewer's Comments: Data are quickly emerging that miRs play important roles in gene regulation, and that depending on its target, a given miR may act either as a tumor suppressor gene or as an oncogene, as the authors point out.

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Easy Method Helps Detect Gout Crystals in FFPE Tissue

Simple Non-Staining Method to Demonstrate Urate Crystals in Formalin-Fixed, Paraffin-Embedded Skin Biopsies.


Using thick, unstained, and cover-slipped sections of formalin-fixed paraffin-embedded tissue enhances detection of gout crystals compared to standard H&E-stained sections.

**Background:** Physical manifestations of tophaceous gout include development of skin nodules that often involve upper and lower digits, as well as the helix of the ear. During bouts of hyperuricemia, monosodium urate (MSU) crystals precipitate in the skin, appearing as white chalky material on gross examination. If fixed in alcohol, these gouty tophi usually appear as brown, negatively birefringent crystals in the dermis or subcutaneous tissue. However, since urate crystals show increased solubility in both formalin and aqueous staining media, characteristic deposits may not be visible in routinely processed sections.

**Objective:** To describe a simple non-staining method to detect and confirm the presence of negatively birefringent crystals using a single thick unstained and cover-slipped slide of formalin-fixed paraffin-embedded (FFPE) tissue.

**Methods:** 29 skin biopsies diagnosed as "gout," "gouty tophus," or "gouty tophi" were retrieved from the pathology archives. For each case, 1 standard 4-m H&E-stained section, 1 unstained and cover-slipped 4-m section, and 1 unstained and cover-slipped 10-m section were prepared. Each case was independently reviewed by 2 dermatopathologists.

**Results:** None of the H&E-stained sections showed needle-shaped crystals or negatively birefringent material using polarization microscopy. Eleven cases (38%) showed diagnostic brown, negatively birefringent needle-shaped crystals on the 4-m unstained sections. Fourteen cases (48%), however, showed definitive MSU crystals using the thicker 10-m sections.

**Conclusions:** Use of a 10-m thick, unstained, and cover-slipped section of FFPE tissue enhances detection of the characteristic brown, negatively birefringent needle-shaped MSU crystals compared to standard H&E-stained sections.

**Reviewer's Comments:** The authors detail prior studies reported by Shidham and colleagues, who document that biopsies <2 mm in greatest dimension or biopsies exposed to formalin for >12 hours are especially susceptible to loss of urate crystals. The simple, straightforward method proposed by the authors seems to be a reasonable first step if crystals are not seen on initial H&E-stained sections.

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ITCs in Breast Cancer SLNs--Displacement or Metastasis?

Isolated Tumor Cells in Breast Cancer Sentinel Lymph Nodes: Displacement or Metastases? An Immunohistochemical Study.

van Deurzen CHM, de Bruin PC, et al:
Hum Pathol; 40 (June): 778-782

Isolated tumor cells in sentinel lymph nodes of breast cancer patients show lower cyclin D1 and p53 expression compared with micrometastases and macrometastases.

Background: Lymph node status is one of the strongest predictors of outcome in patients with breast cancer. Sentinel lymph node (SLN) biopsy is frequently performed because of its accuracy and low morbidity. In some cases, SLNs have very small epithelial cell deposits, classified as isolated tumor cells (ITCs). The clinical significance of these clusters is uncertain. Cyclin D1 and p53 are cell proteins shown to be over-expressed in malignant breast tumors compared to benign breast epithelium.

Objective: To use an ultra-thin sectioning method to evaluate expression of p53 and cyclin D1 in SLN metastases and ITCs.

Participants/Methods: All patients with invasive breast cancer, SLN biopsy, and the finding of ITCs over a 7-year period were included. An additional 15 patients with SLN macrometastases and micrometastases served as controls. Metastatic deposits were classified as ITC if 0.2 mm, micrometastases if >0.2 mm to 2.0 mm, and macrometastases if >2.0 mm. For each patient, 20 serial 1-m thick sections were recut from the SLN block. Every third slide was immunohistochemically stained with AE1/3; blank sections adjacent to the level with AE1/3-positive cells were then stained with p53 and cyclin D1. The percentage of positive nuclei was scored for each case. The primary tumor was also stained for p53 and cyclin D1 for comparison.

Results: 40 patients with ITCs were examined; 16 (40%) revealed ITCs on deeper sections that were amenable to immunohistochemical staining. One additional case showed a micrometastatic focus on deeper sections and was excluded. The median percentage of cells staining with p53 and cyclin D1 in ITCs was 0% (range, 0% to 50%) and 0% (range, 0% to 95%), respectively. In comparison, the median percentage of p53 and cyclin D1 staining for both micrometastases and macrometastases was 1% (range, 0% to 100%) and 75% (range, 0% to 100%), respectively. Expression of p53 and cyclin D1 was significantly lower in ITCs than in other metastases. p53 staining of the primary tumor showed no significant difference from ITCs or other metastases. Cyclin D1 staining of the primary tumor was significantly lower than in micrometastases and macrometastases but was similar to that seen in ITCs.

Conclusions: ITCs in SLNs of breast cancer patients show lower cyclin D1 and p53 expression compared with micrometastases and macrometastases. This supports the hypothesis that these cells may be displaced benign cells or tumor cells that could lack malignant potential.

Reviewer's Comments: This is the first study to evaluate ITCs in breast cancer SLNs using p53 and cyclin D1. It would be interesting to follow these patients and to determine if disease-free survival and overall survival are related to the level of p53 or cyclin D1 expression.

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Manage Proliferative Breast Lesions Without Atypia More Conservatively

Breast Fine-Needle Aspiration Samples Reported as "Proliferative Breast Lesion": Clinical Utility of the Subcategory "Proliferative Breast Lesion With Atypia".
Zhao C, Raza A, et al:
Cancer Cytopathol; 117 (April 25): 137-147

The diagnosis of proliferative breast lesion with atypia on breast fine-needle aspiration is clinically significant, as it is associated with a significantly higher likelihood of malignancy.

Background: Breast fine-needle aspiration (FNA) is very successful in indentifying specific benign and malignant diseases, but its role in the evaluation of proliferative breast lesions is not well defined. The category of proliferative breast lesions on FNA diagnosis on histologic follow-up includes both benign (ductal hyperplasia) and malignant (ductal carcinoma in situ) entities.

Objective: To propose using a qualifier of "with atypia" for a subset of proliferative breast lesions on FNA and to correlate these results with histologic findings.

Methods: Breast FNAs from 1 institution and a cytologic diagnosis of proliferative breast lesion were reviewed. Only cases with histologic follow-up were included. Cases were reclassified as proliferative breast lesion without atypia (PBL) and proliferative breast lesion with atypia (PWA). Criteria for PBL included increased cellularity on the aspirate with cohesive epithelial groups. Criteria for PWA included prior findings and at least 1 of the following: pronounced nuclear overlapping/crowding, loss of cohesion, or nuclear pleomorphism/atypia.

Results: 172 breast FNA cases met inclusion criteria; 120 cases (70%) were reclassified as PBL and 52 (30%) were classified as PWA. There was a significant difference in mean age of patients with PBL (37 years) compared to PWA (44 years) and in the size of targeted lesions (1.9 cm for PBL vs 2.7 cm for PWA). No other clinicopathologic features showed statistical significance. Of 120 cases of PBL, 118 (98%) were benign on histology (70% fibroadenomas) and 2 (2%) were invasive ductal carcinomas. Of 52 cases of PWA, 33 (63%) were benign (30% fibroadenomas) and 19 (37%) were malignant (6 invasive ductal carcinomas, 5 invasive lobular carcinomas, 5 ductal carcinoma in situ, and 3 other malignancies). All 4 breast FNAs from men were classified as PWA, and all 4 showed gynecomastia on follow-up.

Conclusions: PWA was clinically significant, as it was associated with a significantly higher likelihood of malignancy. Fibroadenoma accounted for most benign lesions in both categories.

Reviewer's Comments: It is important to note that the term "proliferative breast lesion with atypia" in breast cytology is not equivalent to the term "atypical ductal hyperplasia on histology," and should not be managed without additional workup. However, at institutions where breast FNA is routine, these findings suggest that those patients with a diagnosis of PBL could be managed more conservatively.

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Clonal Receptor Gene Rearrangements Commonly Found With Sporadic HDS

High Frequency of Clonal Immunoglobulin Receptor Gene Rearrangements in Sporadic Histiocytic/Dendritic Cell Sarcomas.
Chen W, Lau SK, et al:
Am J Surg Pathol; 33 (June): 863-873

Histiocytic and dendritic cell sarcomas are frequently shown to have clonal immunoglobulin receptor rearrangements.

Background: Histiocytic and dendritic cell sarcomas (HDSs) are rare. The malignancies are believed to be derived from phagocytic cells or other accessory cells that partake in antigen presentation to lymphocytes. The tumors represent <1% of nodal hematolymphoid malignancies but also can occur at extranodal sites. Currently, diagnosis of these malignancies is based on histologic and immunophenotypic features. While the tumors are generally believed not to harbor clonal receptor rearrangements, cases have been reported previously with such rearrangements. Some have also reported patients with concurrent follicular lymphomas and HDSs that both showed clonal B-cell immunoglobulin receptor rearrangements.

Objective: To investigate molecular findings of 23 cases of HDS that arose sporadically in patients without previous or concurrent lymphoma diagnoses.

Methods: 23 HDSs were identified from 4 different institutions. Immunohistochemistry was performed with antibodies to CD20, PAX5, Oct2, and BOB.1. All cases were tested for clonal immunoglobulin heavy and kappa-light chain rearrangements. Quantitative polymerase chain reaction (PCR) was performed for the t (14:18) typically seen with follicular lymphomas. In situ hybridization was performed for Epstein-Barr virus early RNA-1. Direct sequence analysis was performed with all cases with clonal immunoglobulin heavy or kappa-light chain receptors.

Results: Cases were from 13 males and 10 females with ages ranging between 8 and 79 years. There were 9 cases of dendritic cell sarcomas (mostly follicular) and 14 cases of histiocytic sarcomas. About half the cases were nodal. Nine cases showed clonal immunoglobulin heavy chain rearrangements with or without kappa-light chain rearrangements, and an additional 2 cases showed only kappa-light chain gene rearrangements. These included 7 histiocytic sarcomas and 4 dendritic cell sarcomas. A single histiocytic sarcoma had a t (14;18) by PCR. All 11 cases with clonal immunoglobulin heavy or kappa-light chain receptors were non-reactive with antibodies to PAX5 and BOB.1, whereas 4 of 7 histiocytic sarcomas were positive for Oct2.

Conclusions: Clonal receptor gene rearrangements are commonly found with sporadic HDSs. This suggests that many of the tumors have B-cell genotypes.

Reviewer's Comments: This study is one of the largest studies investigating the presence of gene rearrangements in these rare malignancies. The results suggest that the tumors may arise from cells having undergone B-cell differentiation.

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LVSI Not Useful Indicator for Low-Risk Breast Cancer

Population-Based Study of Peritumoral Lymphovascular Invasion and Outcome Among Patients With Operable Breast Cancer.

Ejlertsen B, Jensen M-B, et al:
J Natl Cancer Inst; 101 (May 20): 729-735

Presence of lymphovascular space invasion does not significantly alter outcome for patients with otherwise low-risk breast cancers.

Background: Postoperative systemic therapies improve disease-free and overall survival in patients with operable breast cancers. Patients with tumors considered to be low risk, however, are able to forego these therapies as treatment benefits for them tend to be small compared to the overall risk of adjuvant chemotherapy. Although lymphovascular space invasion (LVSI) is considered a prognostic indicator with breast cancer, its relative contribution to prognosis in patients with otherwise low-risk disease remains unclear.

Objective: To examine the prognostic importance of this histologic finding in a large cohort of prospectively diagnosed breast cancers.

Participants/Methods: A national breast cancer database was used to select all women diagnosed with breast cancer without distant metastases at the time of diagnosis who had had complete resections over a 7-year period. All patients were treated consistently with radiation therapy depending on characteristics of their breast cancers. All tumors were classified as to histologic type and grade. Tumor size, margin status, skin and fascia invasion, node status, estrogen and progesterone receptor status, and LVSI status were recorded. To be considered low risk, patients had to be aged >35 years and to have tumors <2 cm without nodal metastases. Tumors had to be hormone receptor-positive and of favorable grade (grade 1 ductal or grade 1 and 2 lobular). Low-risk patients did not receive adjuvant chemotherapy. Follow-up information was gathered.

Results: There were >15,000 patients enrolled in the study, and nearly 2500 were noted to have LVSI. LVSI was associated with positive nodal status, larger tumors, hormone receptor-negative tumors, higher-grade tumors, and ductal histology. Not surprisingly then, LVSI was associated with worse prognosis when analyzed as a single variable. Of >3000 patients with low-risk tumors, only slightly more than 50 were noted to have LVSI. Here, no prognostic importance was found based on LVSI alone. With high-risk patients, LVSI was an important prognostic indicator, however.

Conclusions: LVSI does not have prognostic implications for patients with otherwise low-risk breast cancers. It should thus not be considered sufficient to warrant systemic therapy in and of itself in these cases.

Reviewer's Comments: This is a large and very well-controlled prospective study regarding LVSI with breast cancers. It should help determine management of otherwise low-risk patients for whom LVSI is identified.
Rare Breast Angiosarcomas May Appear After Breast-Sparing Surgery

Angiosarcoma of the Breast: A Clinicopathologic Analysis of Cases From the Last 10 Years.
Wang XY, Jakowski J, et al:
Ann Diagn Pathol; 13 (June): 147-150

Mammary angiosarcomas, although rare, most often occur postradiation, but few arise de novo. Rosen's grading method should be applied to either for prognostic information.

**Background:** While rare, angiosarcomas of the breast are aggressive neoplasms with a poor prognosis compared to conventional carcinomas of the breast. Angiosarcomas can arise de novo, within the field of radiation for primary carcinoma, or from chronic lymphedema after axillary dissection for mammary carcinoma.

**Objective:** To review clinicopathologic features and clinical outcomes of angiosarcomas of the breast from a single institution.

**Methods:** Pathology archives representing 10 years were searched to find 11 breast angiosarcomas. These were then graded by Rosen's method. Clinical information including follow-up was collected for each identified patient.

**Results:** All patients were female, and ranged from age 44 to 94 years, with a median of 66 years. Tumors ranged from 1.6 to 11.1 cm. Eight of 11 patients had received radiation therapy for a prior breast carcinoma, while the remaining 3 had no history of a prior carcinoma. Of postradiation cases, 7 were considered cutaneous and 1 parenchymal. Six of these were high grade, and the remaining 2 were intermediate grade. The time interval from radiation to diagnosis of angiosarcoma ranged from 4 to 12 years. With a median follow-up of 36 months for all patients, skin or chest wall recurrence was documented in 3 of 8 postradiation cases at 5, 9, and 27 months. One of the non-radiation-associated cases developed a liver metastasis 3 years after diagnosis.

**Conclusions:** Angiosarcomas of the breast remain a rare entity despite continued use of radiation therapy after breast-sparing surgery. In this review, limited by small numbers, there are no distinct clinicopathologic differences between de novo and postradiation angiosarcomas.

**Reviewer's Comments:** In the context of radiation exposure for prior breast carcinoma, diagnosis of angiosarcoma may be more readily considered. However, angiosarcoma can be easily confused with a high-grade carcinoma, especially on a limited sample. A panel of immunohistochemical stains could be helpful when vasoformative morphology is absent or equivocal.

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Prior Excision Should Not Supersede Worrisome Features of Recurrent Lesion

Recurrent Nevus Phenomenon: A Clinicopathologic Study of 357 Cases and Histologic Comparison With Melanoma With Regression.

King R, Hayzen BA, et al:

*Mod Pathol;* 22 (May): 611-617

Recurrent nevi and melanoma with regression only rarely overlap in histologic features, but careful review of prior and current material in the clinical context is prudent.

**Background:** Recurrent nevus phenomenon is the development of a recurrent nevus at the site of a prior excision. Given the presence of complicating prior biopsy site changes, the lesion can resemble a melanoma with regression and be misinterpreted. The clinical history of a prior benign lesion having been excised may be forgotten or omitted on submission forms, so the phenomenon is important to consider.

**Objective:** To characterize histologic features and clinical context of cases representing recurrent nevus phenomenon in comparison with malignant melanoma with regression.

**Methods:** All cases of recurrent nevus were retrieved from archived files and were included in the study if the clinical history and histology from both primary and recurrent lesions were available for review. These were reviewed for effacement of the retiform epidermis, junctional and/or dermal melanocytic hyperplasia, growth pattern, relationship of the melanocytic proliferation to the dermal scar, cytologic atypia of melanocytes, and presence or absence of residual nevus. Thirty-four cases of unequivocal malignant melanomas with regression were used as a comparison and defined as follows: early (nests of melanocytes replaced by dense lymphoid infiltrates), intermediate (early fibrosis with lymphoid infiltrate replacing melanocytes), and late (extensive fibrosis and telangiectasia with tumor absence and epidermal effacement).

**Results:** 325 patients were included in the review of recurrent nevi, with an average time to recurrence of 8 months. Patterns recognized included type 1 (junctional melanocytic hyperplasia with effacement of the retiform epidermis), type 2 (compound melanocytic hyperplasia with effacement of the retiform epidermis), type 3 (junctional melanocytic hyperplasia with retention of the retiform epidermis), and type 4 (compound melanocytic hyperplasia with retention of the retiform epidermis). Recurrent nevus with a type 3 pattern resembled melanoma with fibrosis. Melanomas with early or intermediate regression were readily identified by residual malignant melanocytes, but those with late regression overlapped with recurrent nevi having types 1 and 2 patterns.

**Conclusions:** Recurrent nevi have multiple patterns, most of which are readily distinguished from malignant melanoma, especially if the proper clinical context is recognized. Care should be taken in superficial biopsies, in cases with overlapping features, or when clinical history is not available.

**Reviewer's Comments:** Being provided a history of a prior excision should not supersede any worrisome features in a recurrent lesion, and in many cases, review of the original nevus may be prudent.

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