Does FCM Contribute to Dx of Myelodysplastic Syndromes?

The Utility of Flow Cytometric Immunophenotyping in Cytopenic Patients With a Non-Diagnostic Bone Marrow: A Prospective Study.

Truong F, Smith BR, et al:
Leuk Res; 2009; 33 (August): 1039-1046

In morphologically subtle bone marrow biopsies that lack cytogenetic abnormality, flow cytometry facilitates the distinction of MDSs from reactive chronic cytopenias.

Background: Recent investigations indicate that flow cytometry (FCM) contributes to both the diagnosis and risk assessment of myelodysplastic syndromes (MDS). Most myelodysplastic marrows demonstrate abnormal patterns of myelomonocytic staining for CD13, CD16, CD11b, CD14, CD36, or CD15. Some reveal persistent staining for myeloblast markers HLADR and CD34 on maturing granulocytes or aberrant blast staining for CD7 or CD56.

Objective: To prospectively evaluate, with clinical follow-up, the FCM assay for patterns of myelomonocytic maturation as a diagnostic marker of MDS.

Methods: 102 consecutive bone marrow samples without morphologic dysplasia or cytogenetic abnormality that were submitted for evaluation of cytopenias at the University of Massachusetts Memorial Medical Center were evaluated using an antibody panel designed for evaluation of MDS. Samples submitted with clinical concern for lymphoma/leukemia were analyzed by a different panel and were not included in this study. By previous criteria, myelomonocytic maturation was scored for myelodysplasia as follows: negative, <3 abnormal maturing myelomonocytic markers; intermediate, blasts with abnormal CD117 or CD45 staining; abnormal myeloid side scatter, 3 to 4 abnormal maturing myelomonocytic markers; or positive, increased or lymphoid antigen-positive myeloblasts, >5 abnormal maturing myelomonocytic markers.

Results: Cases with leukemias, obvious morphological dysplasias, and cytogenetic abnormalities were excluded. Ages ranged from 22 to 96 years (mean, 66 years), and 19% had suboptimal marrow aspirate morphologic preparations. FCM results were negative in 69%, intermediate in 11%, and positive in 22%. Patients were followed up for a mean of 11 months before being diagnosed by clinical hematologists with one of the following: (1) a stem-cell disorder consistent with MDS; (2) cytopenia due to a non-stem-cell disorder; or (3) cytopenia due to unknown cause. MDS (group 1) patients were positive by FCM in 75% of cases (9 of 12), compared to non-MDS (group 2) patients, who were positive in 7% of cases (4 of 61; \( P < 0.0001 \)). FCM had a positive predictive value of 69% and a negative predictive value of 95%. MDS (group 1) patients were more thrombocytopenic (average, 103,000 vs 197,000), were more macrocytic (97 vs 89), and had less hematogones (0.03% vs 1.1%) than group 2 patients.

Conclusions: In morphologically subtle bone marrow biopsies that lack cytogenetic abnormality, FCM facilitates the distinction of MDS from reactive chronic cytopenias.

Reviewer's Comments: This report is in contrast to many descriptions of FCM findings in MDS by (1) excluding morphologically and genetically obvious cases, and (2) including clinical follow-up of morphologically subtle cases. Among blast abnormalities, identification of a discrete population, decreased CD45, and increased CD117 staining were useful markers of myelodysplasia. Among maturing myeloid abnormalities, abnormal patterns of CD33/CD15 and CD11b/CD13/CD16 were useful. (Reviewer-Guy E. Nichols, MD, PhD).

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Is SALL4 Specific for Metastatic Germ Cell Tumors?

**SALL4 Is a Novel Sensitive and Specific Marker for Metastatic Germ Cell Tumors, With Particular Utility in Detection of Metastatic Yolk Sac Tumors.**

Cao D, Humphrey PA, Allan RW; *Cancer;* 2009; 115 (June 15): 2640-2651

SALL4 is strongly positive in >90% of tumor cells in all metastatic seminomas, dysgerminomas, embryonal carcinomas, and the majority of yolk sac tumors.

**Background:** The accurate diagnosis of metastatic germ cell tumors is critical, as these tumors can be effectively treated with modern therapy. However, germ cell tumors are uncommon, have diverse morphologic features, and can metastasize before recognition of a primary tumor, which complicates an initial diagnosis. Immunohistochemistry facilitates accurate diagnosis; useful markers include placental-like alkaline phosphatase (PLAP), CD30, KIT, alpha-fetoprotein (AFP), and OCT4. Unfortunately, none of these markers is uniformly sensitive or specific, particularly in cases of metastatic yolk sac tumors. Recently, SALL4 was identified as a regulatory transcription factor involved in embryonic stem cells.

**Objective:** To evaluate the utility of SALL4 as a diagnostic immunohistochemical marker for metastatic germ cell tumors.

**Methods:** 73 metastatic testicular germ cell tumors, 13 metastatic ovarian germ cell tumors, and 4 metastatic germ cell tumors of extragonadal sites were evaluated. An additional 170 metastatic somatic tumors from various sites were included to test specificity. Immunohistochemical staining for SALL4 was performed on each case (germ cell and somatic tumors), and staining for OCT4 was also performed on all germ cell tumors. Only nuclear staining was considered positive, and SALL4 staining was semiquantitated as 0 (no tumor cells), 1+ (0% to 30%), 2+ (31% to 60%), 3+ (61% to 90%), and 4+ (>90%).

**Results:** The metastatic germ cell tumors were comprised of 22 seminomas, 7 dysgerminomas, 21 embryonal carcinomas, 15 yolk sac tumors, 7 nongestational choriocarcinomas, and 18 teratomas (2 immature). SALL4 expression was extensive (4+) in 100% of seminomas, dysgerminomas, and embryonal carcinomas. SALL4 expression was 4+ in 14 of 15 (93%) metastatic yolk sac tumor cases and 3+ in the remaining case. Teratomas (both mature and immature) showed rare (1+) staining in 50% and no staining in 50%. Choriocarcinomas showed rare to abundant (1+ to 3+) staining in mononuclear trophoblastic cells in 71% of cases. In comparison, OCT4 was positive in 100% of seminomas, dysgerminomas, and embryonal carcinomas, but was negative in all yolk sac tumors, teratomas, and choriocarcinomas. Among metastatic somatic tumors, only rare (1+) staining was present for SALL4 in 6% of tumors (3 cases of esophageal adenocarcinoma, 6 cases of gastric adenocarcinoma, and 1 case of colonic adenocarcinoma). Notably, it was negative in all hepatocellular tumors.

**Conclusions:** SALL4 is strongly positive in >90% of tumor cells in all metastatic seminomas, dysgerminomas, embryonal carcinomas, and the majority of yolk sac tumors. This marker appears to have a high specificity for germ cell origin, particularly if diffuse (>50%) staining is used as the threshold.

**Reviewer's Comments:** This well-designed study shows SALL4 is a promising immunohistochemical marker with particular utility in the diagnosis of metastatic yolk sac tumors, as these frequently have focal, weak expression of PLAP and AFP. (Reviewer-Deborah J. Chute, MD).

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Familiarize Yourself With Acinic Cell Carcinoma

SkAlovA A, Sima R, et al:

Salivary gland acinic cell carcinomas sometimes show high-grade transformation and behave aggressively.

Background: Acinic cell carcinomas (AciCCs) are uncommon low-grade (LG) malignancies of the salivary glands. As with other salivary gland malignancies (such as adenoid cystic carcinomas, polymorphous low-grade adenocarcinomas, mucoepidermoid carcinomas, myoepithelial carcinomas, and epithelial myoepithelial carcinomas), transformation from a lower-grade tumor to a higher-grade tumor has rarely been reported with this malignancy. It is important for pathologists to recognize these changes, as high-grade (HG) salivary gland malignancies generally portend worse prognoses and require more aggressive therapy, such as radical neck dissections.

Objective: To review a series of AciCCs with HG transformation.

Methods: 9 AciCCs from multiple institutions were identified that were characterized by increased nuclear pleomorphism, distinct nucleoli, increased mitotic activity, and areas of necrosis. Histopathology results and clinical and follow-up information were gathered. Immunohistochemistry was performed with numerous antibodies. Mutational analysis was performed for the TP53 gene. Fluorescence in situ hybridization was performed for the HER-2/neu gene.

Results: Patients included 6 women and 3 men who ranged in age from 43 to 76 years. All tumors occurred in the parotid and ranged in size from 2 to 8 cm. Two patients had previously resected conventional AciCC. Margins were involved in 7 of the 9 cases. In patients presenting with new tumors, HG foci represented 5% to 90% of their tumors. No patients had undergone radiation therapy before the development of their HG tumors. On follow-up, 6 patients developed local recurrences, and 5 patients had lymph node metastases and developed distant metastases. Six patients died of their disease at an average of 4.3 years. Histologically, the HG components were composed of anaplastic-appearing cells with abundant cytoplasm and pleomorphic nuclei. Comedo-type necrosis and stromal desmoplasia were present. Tumors generally showed solid and/or glandular growth. Perineural invasion was seen in 5 cases, and intralymphatic growth in 2 cases. Acinar differentiation could not be demonstrated by hematoxylin and eosin or periodic acid-Schiff stains. HG components showed, on average, 9 times the proliferative activity of the lower-grade components by MIB-1 immunostaining. HG foci showed greater p53 and cyclin-D1 immunostaining as well as strong membranous staining with antibodies to beta-catenin. TP53 gene mutations and HER2/neu gene amplifications were not identified.

Conclusions: AciCCs with HG transformation behave much more aggressively than conventional AciCCs. Given the high propensity for lymph node metastases, the authors suggest that patients with these tumors be treated with neck dissections.

Reviewer’s Comments: This paper presents a large series of AciCCs with HG transformation. Although the condition is uncommon, pathologists should be aware of the phenomenon so that afflicted patients will receive more adequate therapy. (Reviewer-Edward B. Stelow, MD).

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Approximately 15% of atypical glandular cells on Pap test may be premalignant/malignant, but they are not always cervical and not always glandular.

**Background:** Atypical glandular cells (AGCs) on a Pap test can represent a variety of benign and neoplastic lesions of both cervical and noncervical origin. While the sensitivity of a Pap test may be better for squamous lesions than for glandular lesions, AGCs may represent adenocarcinoma in situ, high-grade squamous dysplasia, or invasive carcinomas on histologic follow-up.

**Objective:** (1) To follow a large series of AGCs with histologic follow-up; and (2) to use verification bias-adjusted contextual data on overall sensitivity for the detection of cervical disease and the contribution of AGC as a diagnosis in detecting cervical, endometrial, and adnexal neoplasms.

**Methods:** Pap test results including AGC of endocervical (AGC-EC), endometrial (AGC-EM), or not otherwise specified (ACG-NOS) origin were collected. Excluded were those designated as adenocarcinoma, adenocarcinoma in situ, or suspicious for adenocarcinoma. Those with a coexisting squamous abnormality on Pap test were collected into subsets based on the squamous abnormality. Upon histologic review of follow-up specimens, the neoplasms were categorized into (1) cervical squamous neoplasms, (2) cervical glandular neoplasms, (3) endometrial neoplasms including hyperplasia, or (4) ovarian carcinoma. Laboratory sensitivity was calculated by examining Pap test results during 1 year preceding neoplastic diagnoses, and verification bias was adjusted with findings from 2000 hysterectomy specimens.

**Results:** 1021 Pap tests were reported as AGC, representing 0.41% of all cases. A total of 662 of these had histologic follow-up, and 101 (15.3%) of these were precancerous or malignant neoplasms from the cervix (8.3%), endometrium (6.3%), and ovary (0.6%). AGC with a coexisting squamous lesion or designated AGC-EC tended to have cervical lesions on follow up, and AGC-EM tended to have endometrial lesions on tissue follow-up. The diagnoses of cervical squamous neoplasia or endometrial neoplasia were each more likely after AGC than after finding a cervical glandular neoplasm ($P < 0.001$); however, cervical squamous neoplasia was even more common than endometrial neoplasia ($P < 0.001$).

**Conclusions:** While many examples of AGC on Pap test may be reactive conditions or benign lesions, approximately 15% represent precancerous or malignant lesions from the cervix (including squamous neoplasia), endometrium, or ovary. For the detection of premalignant cervical neoplasia, the verification bias-adjusted screening sensitivity is 93%.

**Reviewer’s Comments:** In women <40 years old, neoplastic lesions were more likely to be cervical compared to those in women >50 years of age, when they were more likely to be endometrial. Thus, HPV testing should not significantly alter follow-up after AGC, especially in women >50 years old. (Reviewer-Mary T. Galgano, MD).

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Features Predict Lung Ca Response to EGFR-TKIs

EGFR and K-ras Mutations Along the Spectrum of Pulmonary Epithelial Tumors of the Lung and Elaboration of a Combined Clinicopathologic and Molecular Scoring System to Predict Clinical Responsiveness to EGFR Inhibitors.

Sartori G, Cavazza A, et al:
Am J Clin Pathol; 2009; 131 (April): 478-789

The combination of gender, smoking history, and mucinous histology predicts lung cancer response to EGFR-TKIs, with or without consideration of EGFR and K-ras mutation status.

**Background:** Among primary lung carcinomas, those with wild type (nonmutated) K-ras genes or with EGFR exon 19 or 21 mutations are most likely to respond to EGFR-TKIs (gefitinib and erlotinib). Clinicopathologic features including a nonsmoking history, female gender, Asian race, and adenocarcinoma histology also predict drug response. Among Korean patients, EGFR mutational status was more predictive of drug response and overall survival than were clinicopathologic features.

**Objective:** To evaluate clinicopathologic features as predictors of lung cancer response to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs).

**Methods:** 418 primary epithelial tumors of the lung obtained from pathology files in Italy were characterized for EGFR exons 18, 19, and 21 mutations and K-ras exon 2 mutations using a previously described direct-sequencing polymerase chain reaction. Clinicopathologic scores ranging from -2 to +5 were assigned based on 1 point each (+1) for EGFR gene mutation, nonsmoking history, female gender, Asian race, and adenocarcinoma histology, and 1 point less (-1) for K-ras mutation and current smoker status.

**Results:** Among 219 men and 199 women, none were Asian; therefore, clinicopathologic scores could range only up to a maximum of 4. Among 418 cases, 286 tumors were adenocarcinomas (68.4%), and 57 (13.6%) demonstrated mucinous features. There were 51 tumors with EGFR mutations (12.2%) and 102 tumors with K-ras mutations (24.4%). Within the subset of 154 patients receiving EGFR-TKIs, 21% had EGFR mutations and 36% had K-ras mutations. Thirty-five percent had low predictive scores (-2, -1); 38% had intermediate predictive scores (0, +1); and 27% had high predictive scores (+2, +3, +4). EGFR mutations were more prevalent in women (21% vs 4%; \( P < 0.0001 \)), in contrast to K-ras mutations, which were more prevalent in men (30.6% vs 17.1%; \( P = 0.001 \)). K-ras mutations were significantly associated with mucin production versus nonmucin production (47% vs 8%; \( P < 0.0001 \)). Predictive scores indicated response to EGFR-TKIs. However, removal of EGFR and K-ras mutation status did not diminish the predictive power of gender, smoking status, and mucinous histology.

**Conclusions:** The combination of gender, smoking history, and mucinous histology predicts lung cancer response to EGFR-TKIs, with or without consideration of EGFR and K-ras mutation status.

**Reviewer's Comments:** Data from this study indicate an association between K-ras mutations and mucinous lung cancers in general, not just bronchioloalveolar. Data also suggest that EGFR mutations are not critical to neuroendocrine tumorigenesis. (Reviewer-Stacey E. Mills, MD).

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How to Treat Patients With MDS

How I Treat Patients With Myelodysplastic Syndromes.

Stone RM:

**Blood; 2009; 113 (June 18): 6296-6303**

MDS patients can be considered for conservative follow up, eligibility for erythropoietin or immunosuppression, transplantation, and DNA-hypomethylating agents.

**Background:** Increasing therapeutic options are driving the need for more accurate diagnosis and risk stratification of MDS.

**Objective:** To recommend diagnostic, supportive, and treatment strategies for patients with myelodysplastic syndromes (MDS).

**Recommendations:** More recent than the International Prognostic Scoring System is the World Health Organization (WHO) classification-based Prognostic Scoring System for MDS, which awards total points ranging from 0 to 6 based on WHO classification (0 for RA, RARS, 5q-; 1 for RCMD, RCMD-RS; 2 for RAEB-1; 3 for RAEB-2), karyotype risk (0 for -Y, 5q-, normal; 1 for all others; 2 for -7 and complex), and transfusion requirement (0 for none; 1 for yes). There are few clinical data on the treatment of secondary disease, which may make secondary MDS irrelevant to the following recommendations. Overall, the majority of primary MDS patients have a 20% to 30% likelihood of responding to therapies. In contrast, the majority of select patients with isolated 5q-syndrome respond to lenalidomide, and most patients with chronic myeloproliferative disease and t(5;12)(q33;p13) involving platelet-derived growth factor receptor-beta respond to imatinib. At some point, most anemic MDS patients undergo a trial of erythropoietin, which is more effective in low-risk patients who have serum erythropoietin levels <500 IU/mL. Rarely, granulocyte-stimulating factor therapy is indicated and effective in some neutropenic patients with infections. The oral iron chelator deferasirox has not yet been proven to benefit MDS patients, but can be considered in low-risk patients expected to receive chronic transfusion support. Younger patients and those who are HLAD15+ may respond to immunosuppressive agents, such as cyclosporine or antithymocyte-globulin. Lenalidomide is not approved for non-5q-patients, but has shown comparable effectiveness to erythropoietin in a recent investigation. Younger patients (<55 to 60 years of age) with higher-risk disease are generally candidates for myeloablative transplantation. Nonmyeloablative transplantation is not well studied, but can be considered in patients up to 72 years of age. After considering eligibility for erythropoietin or immunosuppression, the DNA-hypomethylating agents, azacitidine and decitabine, can be considered for all MDS patients.

**Conclusions:** Following a clinicopathologic diagnosis of MDS and assignment of risk stratification, patients can be considered for conservative follow-up, eligibility for erythropoietin or immunosuppression, transplantation, and DNA-hypomethylating agents.

**Reviewer's Comments:** In practice, most patients will fail initial therapy and require individualized management. Small subsets of patients with isolated 5q- syndrome are likely to respond to lenalidomide and may achieve cytogenetic remission. Almost all other patients should be considered for DNA-hypomethylating agents or clinical trial eligibility. (Reviewer-Guy E. Nichols, MD, PhD).

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New Marker for Mesothelioma

Caveolin-1 Is a Novel Immunohistochemical Marker to Differentiate Epithelioid Mesothelioma From Lung Adenocarcinoma.


Caveolin-1 expression, along with other markers, may be used to help distinguish epithelioid mesothelioma from adenocarcinoma.

**Background:** Based on the likelihood of continuing increases in the number of cases of pleural mesothelioma, distinguishing this rare but aggressive tumor from pulmonary adenocarcinoma will continue to present potential challenges to the practicing surgical pathologist. Although no antibody with perfect sensitivity and specificity has been discovered, positive mesothelioma markers such as calretinin, WT-1, and mesothelin are frequently used in the diagnostic workup of difficult cases. Caveolin-1 (Cav-1), the major protein component of caveolar membranes, has numerous cell functions. Its expression is normally seen in a variety of cell types, including the mesenchymal-derived smooth muscle cell, endothelial cell, and fibroblast. In the lung, Cav-1 is expressed in type I alveolar cells and in visceral mesothelial cells.

**Objective:** To determine the expression of Cav-1 in mesothelioma and pulmonary adenocarcinoma.

**Methods:** 80 cases of epithelioid mesothelioma and 80 cases of pulmonary adenocarcinoma were identified for study. Of the mesothelioma cases, 60 were pleural, 15 were peritoneal, 4 were pericardial, and 1 involved the tunica vaginalis. The pulmonary adenocarcinoma cases were a mixture of subtypes, including papillary, solid, acinar, and bronchioloalveolar. All cases were reviewed by 3 pathologists who classified each case according to the World Health Organization 2004 scheme. Representative sections from each tumor were submitted for immunohistochemical analysis using antibodies to calretinin, D2-40, Wilms' tumor-1 (WT-1), thrombomodulin, mesothelin, vimentin, thyroid transcription factor-1 (TTF-1), napsin-A, carcinoembryonic antigen (CEA), surfactant apoprotein A (SPA), Ber-EP4, and MOC-31. Identification of mesothelioma cases was based on positivity for D2-40 or calretinin, and negativity for CEA, TTF-1, napsin-A, Ber-EP4, SPA, and MOC-31. Identification of adenocarcinoma cases was based on positivity for any 2 of the following: CEA, TTF-1, napsin-A, and/or SPA. Cav-1 staining was also performed on each case. The intensity of staining and the percentage of tumor cell positivity were assessed semiquantitatively.

**Results:** Cav-1 expression (membranous and/or cytoplasmic staining) was present in all cases of epithelioid mesothelioma, with >50% of cases showing expression of Cav-1 in more than half of tumor cells. Six cases (7.5%) of pulmonary adenocarcinoma were focally Cav-1 positive. The sensitivity and specificity rates of Cav-1 expression for epithelioid mesothelioma were 100% and 92.5%, respectively.

**Conclusions:** Cav-1 is a sensitive and specific marker for epithelioid mesothelioma, and it is helpful in distinguishing this tumor from adenocarcinoma of the lung.

**Reviewer’s Comments:** Important next steps include assessing Cav-1 expression in other mesothelioma subtypes and determining the contribution to overall sensitivity and specificity of adding Cav-1 to a panel of currently accepted mesothelioma markers, such as WT-1 and calretinin. (Reviewer-T. David Bourne, MD).
New Panel Screens for Monoclonal Gammopathies

Screening Panels for Detection of Monoclonal Gammopathies.

Katzmann JA, Kyle RA, et al:
Clin Chem; 2009; 55 (August): 1517-1522

Combining serum protein electrophoresis and free light chain analysis provides a good initial diagnostic screening test panel for the detection of most monoclonal gammopathies.

Background: The plasma cell proliferative disorders, which are usually referred to as monoclonal gammopathies, include malignant diseases (multiple myeloma, plasmacytoma, plasma cell leukemia, and Waldenstrom's macroglobulinemia), premalignant conditions (smoldering myeloma and monoclonal gammopathy of undetermined significance), and so-called low-tumor-burden diseases (light chain deposition disease and primary amyloidosis). Various strategies for the laboratory testing of these disorders have been proposed, including the use of serum and urine protein electrophoresis (PEL), immunofixation electrophoresis (IFE), and the more recently introduced quantitative serum free light chain testing (FLC). Current recommendations propose using serum IFE and FLC to screen for most plasma cell proliferative disorders, with the addition of urine IFE if primary amyloidosis is also in the differential diagnosis.

Objective: To assess the sensitivity of various tests and test combinations for detecting plasma cell proliferative disorders.

Methods: Clinical and laboratory data from 5235 patients treated at the Mayo Clinic for a monoclonal gammopathy were initially retrieved. Of these patients, 1877 patients fulfilled inclusion criteria of having serum PEL, IFE, and FLC, as well as urine PEL and IFE performed within 30 days of diagnosis. Abnormal serum PEL was defined by the presence of an M-spike, a fuzzy band, hypogammaglobulinemia (<5.5 g/L), an increased b fraction (>=16 g/L), or an increased alpha 2 fraction (>=15 g/L). Abnormal serum FLC was defined as an abnormal FLC kappa-to-lambda ratio (reference range, 0.26 to 1.65). All subjects were assigned to 1 of 9 disease groups: multiple myeloma, smoldering myeloma, monoclonal gammopathy of undetermined significance (MGUS), plasmacytoma, extramedullary plasmacytoma, Waldenstrom's macroglobulinemia, primary amyloidosis, light chain deposition disease, or POEMS syndrome.

Results: 26 of the 1877 study patients had negative results for all tests. When urine testing was excluded from the full panel, 23 additional patients were lost. When FLC testing was excluded, 30 additional patients' diagnoses went undetected. When urine testing was excluded, 23 additional patients had missed diagnoses. Compared to a panel that included serum PEL, IFE, and FLC, a panel with serum PEL plus FLC missed 58 patients. However, all cases of multiple myeloma, macroglobulinemia, and light chain deposition disease were detected.

Conclusions: The most comprehensive testing panel includes serum PEL, IFE, and FLC along with urine PEL and IFE. A panel with serum PEL plus FLC, however, provides a robust initial diagnostic screen in which the sensitivity may be selectively enhanced with the addition of urine studies and/or serum IFE.

Reviewer's Comments: The authors report various reductions in sensitivity for the detection of MGUS, POEMS, plasmacytoma, and primary amyloidosis by adopting a more limited initial screening panel. As other studies have also shown, eliminating serum IFE testing from the initial screening panel does not appear to decrease testing sensitivity for multiple myeloma, macroglobulinemia, or light chain deposition disease. (Reviewer-T. David Bourne, MD).

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Can Molecular Analysis of Pancreatic Cyst Fluid Predict Malignancy?

Shen J, Brugge WR, et al:
Cancer Cytopathol; 2009; 117 (June 25): 217-227

Molecular analysis of pancreatic cyst fluid can add diagnostic value for the diagnosis of malignant and benign mucinous cysts.

Background: Accurate preoperative diagnosis of pancreatic cysts is critical for proper patient management. The algorithm for the management of patients with small cysts (<3 cm) is largely based on imaging characteristics, fine-needle aspiration (FNA) cytology findings, and cyst fluid analysis for carcinoembryonic antigen (CEA) and amylase. However, these criteria suffer from low sensitivity and specificity. The PathFinderTG is a molecular test for pancreatic cyst fluid that has recently become commercially available.

Objective: To correlate the molecular diagnosis (MDx) provided by this test with a clinical consensus diagnosis (CCD) of various benign and malignant pancreatic cysts.

Participants/Methods: 35 consecutive patients who underwent endoscopic ultrasound (EUS)-guided FNA of pancreatic cysts were studied. The PathFinderTG tests evaluated k-ras gene mutations, loss of heterozygosity (LOH) analysis of 15 genomic foci, and DNA quantity in cyst fluid on each case. The MDx predicts a benign nonmucinous cyst when no abnormalities are present and a benign mucinous cyst when 1 abnormality is present. If a k-ras or LOH mutation is present in >75% of total DNA, then a malignant diagnosis is rendered. A CCD was generated based on EUS findings, histologic diagnosis (if available), cytology, and cyst fluid CEA level. Malignant cysts were defined by malignant histology or cytology. Benign mucinous cysts were defined by histologic confirmation or 2 of the following: thick extracellular mucin without atypical epithelium on cytology, cyst fluid CEA >=192 ng/mL, or EUS features consistent with mucinous neoplasm. Benign nonmucinous cysts were defined by histologic confirmation or 2 of the following: cyst fluid CEA <5 ng/mL, nonmucinous cytology, or EUS features consistent with a serous cyst.

Results: On the basis of the CCD criteria, there were 6 malignant cysts, 15 benign mucinous cysts, and 14 benign nonmucinous cysts. The MDx criteria correlated with 5 of 6 malignant cysts (1 case predicted to be benign nonmucinous), 13 of 15 benign mucinous cysts (2 cases predicted to be benign nonmucinous), and 13 of 14 benign nonmucinous cysts (1 case predicted to be benign mucinous). The one false-negative MDx was an intraductal papillary mucinous tumor with carcinoma in situ that had indeterminate cytology; final histology confirmed a malignant diagnosis. The overall concordance of MDx with CCD was 83% for malignant cysts, 87% for benign mucinous cysts, and 93% for benign nonmucinous cysts.

Conclusions: The overall sensitivity and specificity of detecting a malignant pancreatic cyst with the PathFinderTG test was 83% and 100%, respectively. Molecular analysis of pancreatic cyst fluid can add diagnostic value for the diagnosis of malignant and benign mucinous cysts.

Reviewer's Comments: Molecular tests (k-ras mutation, LOH) may be helpful in clinically managing patients with small pancreatic cysts, particularly those with minimal sample volume and/or those with borderline clinical criteria. (Reviewer-Deborah J. Chute, MD).

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Ovarian Endometrioid Adenocarcinoma—Identifying Molecular Pathways

Pathogenetic Pathways in Ovarian Endometrioid Adenocarcinoma: A Molecular Study of 29 Cases.
Geyer JT, Lopez-Garcia et al:

Low-grade and high-grade ovarian endometrioid adenocarcinomas show different mutations.

**Background:** Ovarian tumors are most often epithelial. Most commonly, they are classified as serous. Recently, researchers have shown that serous tumors appear to follow 2 separate developmental pathways: (1) low-grade carcinomas that develop from borderline tumors, and (2) high-grade carcinomas that develop de novo. Although low- and intermediate-grade ovarian endometrioid tumors are believed to develop from endometriosis, less is known about the molecular pathogenesis of these tumors. Even less is known about high-grade ovarian endometrioid adenocarcinomas.

**Objective:** To investigate the molecular changes seen with a large series of ovarian endometrioid adenocarcinomas.

**Methods:** 29 ovarian endometrioid adenocarcinomas were identified, including 10 grade 1 tumors, 11 grade 2 tumors, and 8 grade 3 tumors. Tumors were classified as per the World Health Organization criteria. Five borderline endometrioid tumors were also included. Immunohistochemistry was performed with antibodies to b-catenin and p53. Mutational data were collected from microdissected tissue for b-catenin, KRAS, and PTEN. Tumors were also tested for microsatellite instability.

**Results:** Patient ages ranged from 21 to 71 years and were similar between the 3 grades. Higher-grade tumors were associated with higher-stage disease. Twelve tumors were bilateral. Endometriosis was seen in 15 cases, and 2 tumors were associated with concurrent borderline tumors. Nuclear b-catenin expression was seen with 14 cases, 3 of which had b-catenin mutations. Immunohistochemical expression was especially seen in tumors with squamous differentiation. Six cases showed strong nuclear immunoreactivity with antibodies to p53. This expression was especially seen with high-grade tumors. KRAS mutations were identified in 5 tumors. These were exclusively seen in grade 2 and 3 tumors. PTEN mutations and microsatellite instability were identified in 2 cases each. Of the borderline tumors, all showed nuclear b-catenin expression, and 7 of 8 had gene mutations. One case had a PTEN mutation. No overexpression of p53 or KRAS mutations was seen. None had microsatellite instability.

**Conclusions:** Some molecular changes are more commonly seen in low-grade ovarian endometrioid adenocarcinomas, and others are more likely associated with high-grade tumors. The authors suggest that these tumors may develop via different molecular pathways.

**Reviewer's Comments:** This paper shows that ovarian endometrioid adenocarcinomas possibly develop through alternative genetic pathways. It will be interesting to see if other studies confirm these findings. (Reviewer-Edward B. Stelow, MD).

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Immunoprofiles of Mucinous Neoplasms of Appendix

**Differential Protein Immunoexpression Profiles in Appendiceal Mucinous Neoplasms: A Special Reference to Classification and Predictive Factors.**

Yoon SO, Kim B, et al:

*Mod Pathol;* 2009; 22 (August): 1102-1112

Mucinous adenocarcinomas of the appendix tend to have more protein alterations than mucinous adenomas, and immunoprofiling may contribute to prognosis.

**Background:** Appendiceal mucinous neoplasms are complicated by controversial classification systems and difficulty in assessing invasion and predicting behavior. Many utilize 2 categories, including low-grade appendiceal mucinous neoplasm and mucinous adenocarcinoma. Others consider a mucinous neoplasm of uncertain malignant potential to be when a low-grade neoplasm has epithelium pushed deeply into underlying tissues (but muscularis mucosae is replaced by fibrosis precluding definitive assessment of invasion), the epithelium has formed cystic gland-like structures in the wall, the margin is positive, or excessive mucin is on the serosal surface.

**Objective:** To histologically classify appendiceal mucinous neoplasms and to evaluate immunoexpression profiles for potential predictive factors.

**Methods:** Clinicopathologic information for 70 appendiceal mucinous neoplasms were retrieved from 3 affiliated hospitals and were classified as adenoma, uncertain malignant potential (UMP), or adenocarcinoma. Immunohistochemistry was performed for 24 proteins that were selected from existing literature on mucinous neoplasms of various organs, representing oncogenic, tumor suppressors, cell-cycle regulators, and mucin proteins.

**Results:** Of the 70 neoplasms, 32 were considered mucinous adenomas, 23 were mucinous neoplasms of UMP, and 15 were mucinous adenocarcinomas. Nine of the 24 markers tested were more likely to be altered in adenocarcinomas than in adenomas, and adenocarcinomas tended to have more of these markers altered than adenomas (5.5 vs 1.4), with neoplasms of UMP falling in between (2.6). Among adenocarcinomas, p53 status corresponded to disease-free survival and overall survival. NF-kappaB status, the total number of altered proteins, and b-catenin loss had marginal impacts on outcome. On multivariate analysis, no protein marker was an independent prognostic factor.

**Conclusions:** Evaluation of immunoexpression profiles of appendiceal mucinous neoplasms may correspond and contribute to classification of appendiceal mucinous neoplasms, including 5+ altered proteins, p53, NF-kappaB, and loss of b-catenin as negative predictive factors.

**Reviewer's Comments:** The authors cite Pai and Longacre for their description of mucinous neoplasm of UMP. A table from that manuscript in 2005 describes cytoarchitectural features of mucinous adenoma, but the proximal margin involved or mucin with epithelium was within the appendiceal wall, but was not clearly invasive, or if there was any uncertainty whether there is epithelium within extra-appendiceal mucin. (Reviewer-Mary T. Galgano, MD).

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Diligent Discussion of the Differential Diagnosis for Malignancies of the Ovary

Ovarian Pathology in Risk-Reducing Salpingo-Oophorectomies From Women With BRCA Mutations, Emphasizing the Differential Diagnosis of Occult Primary and Metastatic Carcinoma.

Rabban JT, Barnes M, et al:

Approximately 7% of patients who undergo risk-reducing salpingo-oophorectomy for BRCA1 or BRCA2 mutations have occult malignancies.

Background: Germline mutations of BRCA1 and BRCA2 genes confer an increased risk for the development of ovarian, tubal, and peritoneal carcinomas. Risk-reducing salpingo-oophorectomies (RRSOs) are now standard treatments for patients with these germline mutations. Among the specimens from these procedures, between 2% and 10% harbor occult malignancies.

Objectives: To investigate the pathologic findings in a series of these specimens and to particularly discuss the differential diagnosis for malignancy with these specimens.

Materials/Methods: 108 RRSO specimens from 61 women with BRCA1 mutations, 47 women with BRCA2 mutations, 2 women with PTEN mutations, and 1 woman with Lynch syndrome were reviewed. Thirty-two additional specimens were studied from women with a strong family history of breast and ovarian carcinoma. Fallopian tubes and ovaries were entirely submitted for histologic examination. Specimens were carefully reviewed for any abnormal findings, and immunohistochemistry was used when necessary.

Results: Nearly 70% of the specimens were from women who had a history of breast cancer. Cytologic atypia in normal ovarian or fallopian tube tissue secondary to chemotherapy was not seen. Of the women with BRCA mutations, 6.5% had occult primary carcinomas. No patients who had strong family histories of disease only had occult carcinomas. Of the primary carcinomas, the majority were from the fallopian tubes. The 2 ovarian carcinomas were serous with solid growth. A single patient had an occult metastasis from her previous lobular breast carcinoma. Tumor cells had abundant foamy cytoplasm believed to be secondary to chemotherapy. Fifty-one percent of the cases had lesions considered to be mimics of primary or metastatic carcinoma. These included mostly hilus cell nodules or hyperplasia and hyperthecosis. However, 5 Brenner tumors and 1 adenomatoid tumor were identified.

Conclusions: Ovaries and fallopian tubes removed prophylactically will occasionally contain occult primary or metastatic carcinomas, and careful histologic examination should be performed with these specimens. Pathologists should also be aware of the many lesions that could potentially mimic these malignancies.

Reviewer's Comments: This paper is exceptionally helpful insomuch as it diligently discusses the differential diagnosis for epithelial malignancies of the ovary. Pathologists who view RRSO specimens may wish to peruse it. (Reviewer-Edward B. Stelow, MD).

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When Should You Order BCR-ABL Kinase Domain Mutation Testing?

Laboratory Practice Guidelines for Detecting and Reporting BCR-ABL Drug Resistance Mutations in Chronic Myelogenous Leukemia and Acute Lymphoblastic Leukemia: A Report of the Association for Molecular Pathology.

Jones D, Kamel-Reid S, et al.

J Mol Diagn; 2009; 11 (January): 4-11

Acquired mutations in the BCR-ABL kinase domain predict likelihood of response to second-generation kinase inhibitors. Some confer high resistance, while others may be managed simply by increasing drug dose.

Background: The BCR-ABL t(9;22)(q34;q11) translocation triggers malignant transformation in chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL). BCR-ABL translocation products also serve as the targets for first-line therapies such as Gleevec, that competitively inhibit BCR-ABL kinase function. Standard-of-care Gleevec therapy includes reverse transcription quantitative polymerase chain reaction (RQ-PCR) measurement of BCR-ABL RNA every 3 to 6 months. Mutations in the BCR-ABL kinase domain are major, but not exclusive, factors in acquisition of Gleevec resistance.

Objective: To review current laboratory practice for the measurement and monitoring of BCR-ABL transcripts and mutations, and to recommend practice guidelines for test indications, methodology, and reporting.

Review: Clinicians should consider ordering BCR-ABL kinase domain mutation analysis in CML patients who do not respond initially to Gleevec and in those who respond but develop molecular relapse, which is not universally defined, but is considered by some as a 10-fold increase in BCR-ABL RNA. An international panel of experts recommends the Sanger direct sequencing method, which detects 1 mutated BCR-ABL transcript among 5, although some labs employ more sensitive mutation RQ-PCR or pyrosequencing mutation screening methods. Common BCR-ABL kinase domain mutations cluster to 4 regions, and 7 mutated codons comprise 60% to 70% of mutations. Some mutations (eg, T315I) confer high resistance, while some low resistance mutations (eg, M351T) may be managed simply by increasing drug dose. The use of second generation kinase inhibitors like dasatinib and nilotinib is already associated with specific acquired mutations. To assess the varied collection, RNA extraction, reverse transcription, and mutation analysis methods, future development of testing standards will require reference material and proficiency testing.

Conclusions: Many acquired mutations in the BCR-ABL kinase domain predict the likelihood of response to second-generation kinase inhibitors. However, future development of testing standards and expanded sharing of clinical data are required to improve clinical application of BCR-ABL drug resistance monitoring.

Reviewer's Comments: Not all CML patients develop drug resistance through BCR-ABL kinase domain mutations. Some acquire separate chromosomal abnormalities, nonkinase domain BCR-ABL mutations, gene amplification, other pathways of BCR-ABL protein overexpression, or altered drug metabolism. Not all BCR-ABL kinase domain mutations arise following drug therapy, since some can be detected by PCR in untreated patients. Not all BCR-ABL mutations are point mutations, as some arise from alternate splicing, gene insertions, and deletions or duplications (Reviewer-Guy E. Nichols, MD, PhD).

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Effective Detection of Minimal Residual Multiple Myeloma


Harrington AM, Hari P, Kroft SH:

Paraffin CD56 immunostaining effectively detects minimal residual multiple myeloma in posttherapy bone marrow biopsies.

Background: Roughly 75% of multiple myelomas aberrantly express surface CD56. The 2006 International Myeloma Working Group criteria for myeloma therapeutic response apply bone marrow histology, electrophoresis/immunofixation, and serum free light chain ratios to define complete responses, stringent complete responses, very good partial responses, and partial responses. Complete and stringent complete responses require <5% marrow plasma cells with the absence of plasma cell light chain restriction by flow cytometry or immunohistochemistry. The application of CD56 immunohistochemistry to monitoring of disease has not been characterized.

Objective: To evaluate paraffin CD56 immunohistochemistry as a monitor of multiple myeloma response to therapy.

Methods: Post-therapy bone marrow aspirate smears and Bouin-fixed biopsies from 111 plasma cell neoplasia patients followed at the Medical College of Wisconsin were retrospectively reviewed. Plasma cell clusters contained >2 plasma cells. Kappa:lambda ratios >4 or <0.5 constituted light chain restriction. Plasma cell cytoplasmic light chain staining was evaluated by flow cytometry by gating on CD38-bright events.

Results: Among 20 controls (negative lymphoma staging samples), 11 (55%) demonstrated a small number of benign-appearing, weak-intensity CD56+ plasma cells, averaging 1.2% of total cells. Among 127 postmyeloma therapy bone marrows, 74 (58%) were positive for residual myeloma by conventional morphologic and/or laboratory criteria, averaging 18% plasma cells. In contrast, 53 negative biopsies averaged <1% plasma cells. Among the 74 myeloma-positive samples, 80% contained CD56+ plasma cells by immunostaining. Among these CD56+ samples, flow cytometry was 80% sensitive in detecting CD56+ plasma cells, which averaged 1.8% of total flow cytometry events. Among the 53 negative samples, 3 contained few CD56+ morphologically atypical plasma cells, compatible with minimal residual disease even though light chain restriction was not confirmed.

Conclusions: Paraffin CD56 immunostaining effectively detects minimal residual multiple myeloma in posttherapy bone marrow biopsies.

Reviewer's Comments: There is some controversy in the literature regarding CD56 staining in monoclonal gammopathy of undetermined significance and benign marrows. This study confirms that there can be low number, low intensity CD56 plasma cell staining in benign marrows. Morphologic correlation is required to distinguish CD56+ natural killer cell and large granular lymphocytes. (Reviewer-Guy E. Nichols, MD, PhD).

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Saliva DNA May Have Place in Detection of HNSCC

Noninvasive Molecular Detection of Head and Neck Squamous Cell Carcinoma: An Exploratory Analysis.

The combination of 2 genes, PMAIP1 solely or in conjunction with PTPN1, from saliva DNA is able to differentiate HNSCC patients from normal controls.

Background: Head and neck squamous cell carcinoma (HNSCC) has a poor 5-year survival, despite advances in treatment. One explanation for the continued poor outcomes is late detection. Early detection through clinical screening has been attempted in the past, but has had limited utility. Molecular analysis of HNSCCs has shown that malignant phenotypes typically contain a number of genetic alterations; however, no single mutation is sufficient in predicting disease course. High throughput molecular assays, such as multiplex ligation-dependent probe amplification (MLPA), can permit detection of alterations in a large number of gene targets and potentially identify HNSCC-gene alterations.

Objective: To analyze saliva for HNSCC-specific genetic alterations using MLPA.

Participants/Methods: 27 patients with HNSCC and 10 normal controls were studied. HNSCC tumors were located in the larynx (8), oral cavity (7), tonsils (5), oropharynx (2), and pyriform sinus (1); the final 4 patients had unknown primary sites with neck metastasis. Two mL of saliva were collected from each study subject in a noninvasive fashion. Saliva DNA was tested by MLPA for a total of 82 genes. A relative copy number of each gene probe was normalized to the control, and semiquantified as normal (0.75 to 1.3), loss (<0.75), or gain (>1.3). The gene results were analyzed using the classification and regression tree (CART) statistical tool to generate a gene-based discriminatory algorithm.

Results: CART analysis identified 2 gene probes (PMAIP1 and PTPN1) that classified study subjects correctly. If there was a gain of PMAIP1, study subjects were correctly classified as part of the HNSCC group. If there was a normal or a loss in both PMAIP1 and PTPN1, study subjects were correctly classified as normal controls. The estimated sensitivity and specificity of these combined markers were 96.3% and 80%, respectively, based on leave-one-out validation of this algorithm.

Conclusions: This study demonstrates that saliva genomics may have clinical utility for noninvasive HNSCC detection and screening. The combination of 2 genes, gain of PMAIP1 or in conjunction with gain of PTPN1, from saliva DNA can differentiate HNSCC cases from normal controls.

Reviewer's Comments: This preliminary study identified promising molecular markers of HNSCC. However, further studies are needed with larger patient cohorts and patients with preneoplastic disease and/or high-risk factors to determine whether this will have clinical utility for screening or disease detection. (Reviewer-Deborah J. Chute, MD).

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ImmunoCyt Shows Highest Sensitivity for Detecting Low-Grade Urothelial Tumors

Comparison of ImmunoCyt, UroVysion, and Urine Cytology in Detection of Recurrent Urothelial Carcinoma: A “Split-Sample” Study.

ImmunoCyt has a higher sensitivity, but a lower specificity, for urothelial neoplasia than UroVysion.

**Background:** Patients with superficial urothelial carcinoma can be treated with bladder-preserving therapy, but remain at increased risk for recurrence. The current recommendation is to undergo frequent cystoscopic surveillance with urine cytology to detect new, recurrent, or persistent disease. However, cystoscopy is invasive, expensive, unpleasant, and suffers from low sensitivity. Ancillary tests to assist with urine evaluation have been developed: ImmunoCyt and UroVysion have been independently shown to have utility as adjuncts to urine cytology.

**Objective:** To compare these 2 techniques with urine cytology to determine the best method for detecting low-grade recurrent urothelial carcinoma.

**Participants/Methods:** Voided urine samples were obtained from 100 patients currently undergoing surveillance for urothelial carcinoma recurrence. Cystoscopy was performed after urine collection, and any suspicious lesions were biopsied. The urine samples were split into 3 equal parts according to the required volumes for UroVysion, ImmunoCyt and liquid-based cytology. ImmunoCyt samples were considered positive when at least 1 green or red fluorescent urothelial cell was identified, according to the manufacturer's directions. UroVysion samples were considered positive if >=4 cells showed gains of 2 or more chromosomes or if >=12 cells showed a homozygous deletion of 9p21. Cytology samples were classified as negative, atypical, or positive for malignancy. For calculation of sensitivity and specificity, cytology cases with an atypical diagnosis were combined with the negative category.

**Results:** 35 patients had abnormalities at cystoscopy that were biopsied; of these, 25 were malignant (13 low-grade urothelial carcinomas, 11 high-grade urothelial carcinomas, and 1 not classified). Urine cytology classified 63 cases as negative, 28 as atypical, 7 as positive, and 2 as inadequate for evaluation. ImmunoCyt classified 52 cases as negative, 46 as positive, and 2 as inadequate. UroVysion classified 79 cases as negative, 9 as positive, and 12 as inadequate. Cytology, ImmunoCyt, and UroVysion detected 15%, 62%, and 8% of low-grade tumors, respectively. The overall sensitivity and specificity for urothelial neoplasia (using histology as the gold standard) was 21% and 97%, respectively, for cytology, 76% and 63%, respectively, for ImmunoCyt, and 13% and 90%, respectively, for UroVysion.

**Conclusions:** ImmunoCyt showed the highest sensitivity for detecting low-grade urothelial tumors, but lacked the specificity of urine cytology or UroVysion. Because of this, ImmunoCyt likely will have the best utility as an ancillary test to urine cytology.

**Reviewer's Comments:** It is important to note that this study was focusing on the detection of low-grade tumors, as both urine cytology and UroVysion are known to have decreased sensitivity for these lesions. Whether clinical intervention and treatment would be recommended for patients with recurrent/persistent low-grade disease is unclear. (Reviewer-Deborah J. Chute, MD.)

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MCC--Another Tumor Associated With a Viral Pathogenesis

Clinical Factors Associated With Merkel Cell Polyomavirus Infection In Merkel Cell Carcinoma.
Sihto H, Kukko H, et al:
J Natl Cancer Inst; 2009; 101 (July 1): 938-945

Most Merkel cell carcinomas of the skin have integrated merkel cell polyomavirus.

**Background:** Merkel cell carcinoma (MCC) is an uncommon neuroendocrine carcinoma of the skin. Compared to other carcinomas of the skin, it has a poor prognosis and the 5-year relative survival for patients with this tumor is approximately 50%. Sun exposure has long been considered the primary etiologic agent for the development of MCCs, although, and of note, immunocompromised patients have been noted to be at increased risk for the tumors. Recently, a novel polyomavirus has been identified in the tumors (Merkel cell polyomavirus [MCPyV]). The virus has been shown to be clonally integrated into the genomes of MCCs. Many now suggest that this virus plays a role in the development of these tumors.

**Objective:** To examine a large series of MCCs for viral integration and its possible relationship with patient outcomes.

**Methods:** A single country's cancer registry was searched for all cases of MMC seen over approximately a 25-year period. Of the 207 cases identified, 93 cases were excluded for various reasons. Tumors were required to be immunoreactive with antibodies to cytokeratin 20 (CK20) or synaptophysin and chromogranin and not to be immunoreactive with antibodies to thyroid transcription factor 1. Tumors were classified based on World Health Organization (WHO) criteria. Clinical and follow-up data were retrieved. MCPyV was detected using polymerase chain reaction and paraffin-embedded, formalin-fixed tissue. Virus status was compared to clinical and outcome parameters.

**Results:** 80 of the 114 patients were men, and the median age was approximately 80 years. Only 8 patients were considered immunocompromised. Approximately equal numbers of cases occurred in the head and neck area and at other sites. The majority of tumors had an intermediate-cell type rather than a small-cell type. Approximately 20% of patients had metastases. Nearly 80% of the cases had detectable MCPyV. The amount of viral DNA compared to control DNA varied from 0.0003 to 4334. The majority of these had ratios \( \geq 1 \). Tumors with viral DNA were more likely than those without viral DNA to be located on the limbs and were less likely to have regional lymph node metastases. Twenty-five patients died due to their disease. Patients with tumors associated with the virus had a better 5-year survival rate than patients who had tumors that were not associated with the virus.

**Conclusions:** Viral infection is frequently seen with MCCs, and it may be associated with clinical outcome.

**Reviewer's Comments:** MCC is yet another tumor associated with a viral pathogenesis. This knowledge may lead to new diagnostic and treatment possibilities. (Reviewer-Edward B. Stelow, MD).

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SETTLE

Spindle Epithelial Tumor With Thymus-Like Differentiation: A Morphologic, Immunohistochemical, and Molecular Genetic Study of 11 Cases.

Thyroid SETTLEs do not harbor synovial sarcoma translocations.

**Background:** Spindle epithelial tumor with thymus-like differentiation (SETTLE) is an extremely rare tumor of the thyroid gland, with <25 cases having been reported. The tumor most often occurs in children and young adults and is associated with uncommon and late lymph node, lung, or other visceral metastases. As the tumor is biphasic and occurs in younger patients, it can sometimes be difficult to distinguish this tumor from synovial sarcoma.

**Objective:** To examine a series of SETTLEs and compare clinical, morphologic, immunohistochemical, and cytogenetic findings with those of synovial sarcomas.

**Methods:** 11 previously diagnosed cases of SETTLE and 2 of "possible SETTLE" were identified from multiple institutions. Clinical and follow-up information were pursued, and immunohistochemistry was performed with numerous antibodies. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed for identification of the SS18/SSX1 and SS18/SSX2 fusion genes typically seen with synovial sarcomas. Fluorescent in-situ hybridization (FISH) was performed for SYT rearrangements.

**Results:** All 10 cases tested previously and diagnosed as SETTLE were found not to have synovial sarcoma-type translocations by RT-PCR and/or FISH. Both cases diagnosed as "possible SETTLE" showed synovial sarcoma-type translocations. Synovial sarcoma had been included in the differential diagnosis of these tumors. Of the 11 SETTLEs, 7 were from females. Ages ranged from 7 to 50 years, with a median age of 13.5 years. All but 1 case involved the thyroid gland. All tumors were believed to be entirely resected. Only 4 cases had follow-up information, and 1 of these eventually developed lung metastases. Tumors were infiltrative and moderately cellular with admixtures of fascicular, reticular, and microcystic zones. These areas blended into epithelial areas with glomeruloid structures, sertoli-like tubules and small glands. Neoplastic cells had elongated nuclei with fine chromatin and indistinct nucleoli. Mitotic figures were rare and necrosis and cytologic atypia were not present. Mast cells were not present although some degree of wiry collagen was frequently seen. Spindled and epithelial components frequently expressed high molecular weight cytokeratins. Epithelial membrane antigen expression was limited to rare spindled or epithelial cells in 4 of the 8 cases tested. Strong membranous expression of bcl-2 and CD99 was seen in most cases as was expression of CD117. Nuclear expression of TLE1 protein was seen in 1 case.

**Conclusions:** SETTLEs are distinct, low-grade, biphasic malignancies that need to be distinguished from synovial sarcomas. Morphologic and immunohistochemical studies can distinguish the tumors in most cases although molecular testing of FISH may sometimes be needed.

**Reviewer's Comments:** This paper describes the pathologic features of a series of thyroid SETTLEs. Surgical pathologists who see thyroid tumors may wish to review it to become familiar with these tumors. (Reviewer-Edward B. Stelow, MD).

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Does Ki-67 Proliferation Index Add Predictive Value to the Oncotype DX Assay?

Complementary Value of the Ki-67 Proliferation Index to the Oncotype DX Recurrence Score.

Gwin K, Pinto M:


The authors propose determining the Ki-67 proliferation index for all cases of breast carcinoma classified as low-risk and intermediate-risk by the Oncotype DX assay.

**Background:** Oncotype DX is a Food and Drug Administration-approved test that measures the expression of a panel of 21 genes in paraffin-embedded breast tissue tumor samples. Randomized studies and prior validation studies have shown that the test predicts clinical benefit from adjuvant chemotherapy in patients with estrogen receptor-positive breast tumors, who do not have evidence of lymph node metastasis. The results of the Oncotype DX test are reported as a recurrence score (RS), and it has been shown that patients with a high-risk RS are more likely to benefit from adjuvant chemotherapy than those patients with a low RS. Although patients with low-risk RSs have an estimated recurrence risk of 7%, no studies have identified which patients with low RSs have an actual risk of tumor recurrence. A high Ki-67 proliferation index has been associated with unfavorable outcomes, and the Oncotype DX panel does include analysis of the Ki-67 gene. However, its expression is factored into the RS itself and is not reported separately.

**Objective:** To determine the association between Ki-67 expression by immunohistochemistry and various other factors, including tumor morphology, tumor grade, and the Oncotype DX RS.

**Materials/Methods:** 32 consecutive cases of invasive breast carcinoma that had been submitted for Oncotype DX testing were retrieved from the pathology archives and reviewed by 2 pathologists. Cases included ductal carcinoma (21), lobular carcinoma (4), mucinous carcinoma (3), and tubular carcinoma (4). A majority of the specimens consisted of lumpectomies (26). All tumors were assigned grades based on the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system. All cases had been previously submitted for estrogen receptor (ER), progesterone receptor (PR), and HER2/neu testing by immunohistochemistry; these slides were also reviewed and their results confirmed. An additional slide of unstained tissue was submitted for Ki-67 staining. This tissue section was submitted from the same block used for Oncotype DX testing.

**Results:** The mean RS was 18.8 (range, 4 to 30), and the mean Ki-67 proliferation index was 23.7% (range, 3.6% to 66.0%). Thirteen cases had low-risk RSs and 19 cases had intermediate-risk RSs. No cases in the study were classified as high-risk by the Oncotype DX assay. In some low-risk RS cases, the Ki-67 proliferation index was unexpectedly high. In general, the RS and Ki-67 index correlated with both tumor type and tumor grade, although the range of Ki-67 proliferation indices was wide.

**Conclusions:** Given that a certain percentage of low-risk and intermediate-risk tumors by Oncotype DX testing also show high Ki-67 proliferation indices, the Ki-67 proliferation index should be assessed in these groups in order to identify patients with increased recurrence potential who might benefit from further chemotherapy.

**Reviewer's Comments:** Although the authors’ conclusions are reasonable, results of larger studies including high-risk Oncotype DX cases are required before adopting their recommendations. (Reviewer-T. David Bourne, MD).

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A Better Method for Detecting Bacterial Contamination in Platelets

Novel Flow Cytometry-Based Screening for Bacterial Contamination of Donor Platelet Preparations Compared With Other Rapid Screening Methods.

Dreier J, Vollmer T, Kleesiek K:
Clin Chem; 2009; 55 (August): 1492-1502

The BactiFlow flow cytometer is useful for detecting bacterial contamination of PLTs.

Background: Both the prevention and detection of bacterial contamination of platelet preparations (PLTs) before transfusion remains a significant challenge. Although the allowable storage time for PLTs was reduced to 5 days many years ago, there is still a significant risk of transfusion-related bacterial infection. This is despite more recent efforts at performing either pathogen reduction procedures or pretransfusion bacterial screening of platelet units. Current rapid approaches to bacterial screening have included fluorescence-activated cell sorting (FACS), qualitative immunoassay, and nucleic acid amplification techniques (NAT).

Objective: To present a new flow cytometry-based pretransfusion bacterial detection method using the BactiFlow instrument and to compare this optimized protocol to other rapid detection methods.

Methods: Apheresis-derived single donor platelets were used for the study. Eleven bacterial strains, including Bacillus cereus, Bacillus subtilis, Clostridium perfringens, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Propionibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, and Yersinia enterocolitica, were used. Bacteria from each strain were inoculated into sterile platelet samples, which were then serially diluted to achieve final concentrations of 102 and 103 CFU/mL. These units then underwent standard processing and storage between 0 and 5 days at 20 degrees C to 24oC with agitation. A total of 214 platelets were then analyzed using both the BactiFlow assay and the BacT/Alert3D continuous monitoring system. After filtering platelet cell debris, the BactiFlow assay was used to measure fluorescence at 540 nm (FL1) and 590 nm (FL2). Microorganisms exhibit a specific FL1/FL2 ratio of approximately 1.0 (range, 0.8 to 1.2). These fluorescence events were recorded as counts/mL. In addition to the above methods, rapid detection of bacteria was also performed using NAT and qualitative immunoassay.

Results: A relatively simple protocol taking <1 hour for screening of platelet concentrates for bacteria was developed using enzymatic digestion followed by centrifugal filtration of viable platelets and fluorescent labelling of bacteria. The BactiFlow assay showed a strong positive correlation with traditional bacterial culture plate counts (r >0.97). The lower limit of detection of the assay was 150 CFU/mL, which is far below what is thought to be a clinically significant level of contamination (105 CFU/mL).

Conclusions: The BactiFlow assay is another option for the rapid detection of bacteria in PLTs. The assay is simple and cost-effective, and it shows acceptable sensitivity and specificity compared with other detection systems.

Reviewer’s Comments: The authors correctly observe that no single method can eliminate the risk of bacterial sepsis caused by transfusion of contaminated platelet products. (Reviewer-T. David Bourne, MD).

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PDGFRA Mutated in Inflammatory Fibroid Polyps

Gain-of-Function PDGFRA Mutations, Earlier Reported in Gastrointestinal Stromal Tumors, Are Common in Small Intestinal Inflammatory Fibroid Polyps. A Study of 60 Cases.


Small intestinal IFP may be PDGFRA-driven neoplasms, but should not be confused with epithelioid GISTs with similar mutations.

**Background:** Inflammatory fibroid polyps (IFP) typically consist of an intraluminal, but submucosal proliferation of fibrovascular tissue with a prominent inflammatory infiltrate, characteristically consisting of numerous eosinophils. While the etiology of these lesions has been debated, IFP of the stomach have recently been demonstrated to express platelet-derived growth factor receptor (PDGFRA) as well as contain oncogenic mutations in the PDGFRA. The PDGFRA gene is highly homologous to KIT, and has been reported to be mutated in a subset of gastrointestinal stromal tumors (GISTs) of the stomach.

**Objective:** To evaluate a series of small intestinal IFP for PDGFRA expression and mutation.

**Materials/Methods:** 60 IFP of the small intestines were collected from the archived files of the Armed Forces Institute of Pathology and immunostained for 2 antibodies for PDGFRA (SC338 and MAB 322) and DOG-1, then compared to prior published results of KIT (CD117), SMA, CD34, and desmin. Tumor DNA obtained from formalin-fixed paraffin-embedded tissue blocks was screened for activating mutations in PDGFRA.

**Results:** 54 (90%) of the IFP had strong, diffuse (n=51) or focal (n=3) positivity for both PDGFRA antibodies. One of the 2 antibodies was positive in an additional 3 cases, while the remaining 3 cases were negative for both. All cases were negative for KIT, DOG-1 and desmin, while 2 had some SMA staining, and 14 had some CD34 staining. Of the 60 tumors (55%), 33 were determined to have a PDGFRA mutation, similar to the pathogenetic gain-of-function mutations demonstrated in GISTs.

**Conclusions:** Small intestinal IFP express PDGFRA and contain activation mutations similar to those demonstrated in the gastric IFP, providing evidence for a neoplastic PDGFRA-driven process.

**Reviewer's Comments:** Given the 95% rate of protein expression, further studies on fresh tissue and evaluating additional mutational sites of the PDGFRA gene may increase the rate of detecting activating mutations. All cases of IFP were negative for characteristic GIST markers (KIT and DOG-1), and these lesions should not be confused with GISTs that harbor a functional PDGFRA mutation. (Reviewer-Mary T. Galgano, MD).

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**Novel 5-Antibody Panel May Increase Diagnostic Yield**

*A Novel Five-Antibody Immunohistochemical Test for Subclassification of Lung Carcinoma.*

Ring BZ, Seitz RS, et al:

*Mod Pathol; 2009; 22 (August): 1032-1043*

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Targeted therapy for lung cancer requires subclassification, which may be done with a 5-antibody panel that is more successful than a TTF-1/p63 panel.

**Background:** Bronchogenic carcinomas have recently been subjected to gene expression with different profiles found in various subtypes. While current therapy does not require differentiation of adenocarcinoma from squamous cell carcinoma, emerging targeted therapy has varying efficacy and toxicity in the different subtypes. Future therapeutic strategies will likely require more precise classification of lung carcinoma, which is not always straightforward on simple hematoxylin and eosin (HE)-based histology, especially given current trends for smaller biopsy samples. Current strategies include immunohistochemistry for thyroid transcription factor-1 (TTF-1) as a marker of glandular differentiation, and p63 as a marker of squamous differentiation, but these overlap and are not always definitive.

**Objective:** To develop an antibody panel to distinguish bronchogenic adenocarcinoma from squamous cell carcinoma.

**Materials/Methods:** 551 patient tumor samples were utilized from multiple tissue microarrays to perform immunohistochemistry targeting a large number of proteins for correlation to histologic types. A model was built for the discrimination of adenocarcinoma from squamous cell carcinoma based on the histologic classification and immunohistologic results.

**Results:** 105 antisera were evaluated and 6 clusters were identified by kappa means clustering that largely corresponded to histologic classification. From these sets, linear regression model selected for squamous markers (SLC7A5, CK5/6, TRIM29) and adenocarcinoma markers (MUC1, CEACAM5). Using this 5-panel classification system, 3% of samples called adenocarcinoma by histology were classified as squamous, and 3% of samples called squamous by histology were classified as adenocarcinoma. Twelve percent of cases were unclassifiable by the established panel of 5 markers. For validation, an additional 1111 samples from various institutions were stained with the 5-antibody panel for strong association with the histologic diagnosis \( P < 0.0001 \). In the validation cohort, the unclassifiable cases ranged from 6% to 12%, and the misclassification rate ranged from 4% to 5%. Comparison to a TTF-1/p63 panel determined similar misclassification rates of 2% to 5%; however, the unclassifiable rate was 14% to 37%. Both panels were able to distinguish many cases that were histologically ambiguous, with 94% agreement between the panels, but a histologic diagnosis of large-cell carcinoma was associated with unclassifiable antibody results by both panels.

**Conclusions:** A novel 5-antibody panel may increase diagnostic yield over a panel including TTF-1 and p63 for distinguishing bronchogenic adenocarcinoma from squamous cell carcinoma.

**Reviewer's Comments:** Aspiration biopsies for cell block and needle core biopsies of lung tumors can be extremely difficult to accurately classify. Clinical importance may emerge as targeted therapy develops and become first-line treatment in bronchogenic carcinoma. (Reviewer-Mary T. Galgano, MD).

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