Alcohol Fixation of Cell Block Preps May Alter IHC Results

Immunohistochemical Detection of Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor 2 Expression in Breast Carcinomas: Comparison on Cell Block, Needle-Core, and Tissue Block Preparations.
Hanley KZ, Birdsong GG, et al:

In cases of breast carcinoma, estrogen receptor immunohistochemistry (IHC) showed high concordance on cell block staining, but progesterone receptor and HER2 IHC showed significant discordant results.

Background: The prognostic and therapeutic implications of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2) status in breast carcinomas are well established. The detection of ER, PR, and HER2 expression has been standardized for formalin-fixed, paraffin-embedded tissue sections. However, the detection of these markers has had variable success in fine-needle aspiration (FNA) specimens.

Objective: To evaluate the accuracy of ER, PR, and HER2 immunohistochemical (IHC) expression on cell block (CB) material which was alcohol-fixed in comparison with needle-core (NC) preparations.

Methods: 41 patients with a diagnosis of primary or metastatic breast carcinoma by FNA and NC were included in the study. CB samples were initially fixed in 50% ethanol and followed by fixation in 10% neutral buffered formalin. IHC was performed with antibodies for ER, PR, and HER2. Using the PathVysion® HER2 DNA probe kit, fluorescent in situ hybridization (FISH) for HER2 was used as a gold standard with an equivocal result on either CB or NC. Nuclear staining in >10% of tumor cells was considered positive for ER and PR. HER2 IHC was scored as follows: 0 to 1+ indicated negative, 2+ indicated equivocal, and 3+ indicated positive.

Results: ER expression was concordant in 88% of cases (5 cases ER-negative on CB and ER-positive on NC). PR expression was concordant in 73% of cases (9 cases PR-negative on CB and PR-positive on NC, 2 cases PR-positive on CB and PR-negative on NC). HER2 expression by IHC was concordant in 85% of cases. HER2 IHC on CB showed 15 cases were positive, 8 cases were equivocal, and 14 cases were negative, while on NC, 8 cases were positive, 14 cases were equivocal, and 19 cases were negative. All CB equivocal cases were negative for HER2 amplification by FISH. Six discrepant cases were equivocal on NC and positive on CB; 3 of these cases were amplified by FISH. Conclusion: Alcohol fixation during cell block preparation does not affect ER results in breast carcinoma, but may alter PR and HER2 IHC results. A significant number of cell block specimens had false-negative PR results and false-positive or equivocal HER2 results by IHC.

Reviewer's Comments: Tissue fixation type and duration is critically important in the evaluation of IHC staining. Subsequent NC or tissue biopsy IHC may be required to appropriately triage patient care.

Additional Keywords: None

Print Tag: Refer to original journal article
CD163 Useful New Marker for Identifying AFX

Diagnostic Value of CD163 in Cutaneous Spindle Cell Lesions.
J Cutan Pathol 2009; 36 (August): 859-864

CD163 should probably be included in the workup of cases in which atypical fibroxanthoma is in the differential diagnosis.

Background: Atypical fibroxanthoma (AFX) is a cutaneous mesenchymal tumor with a strong resemblance to malignant fibrous histiocytoma/pleomorphic sarcoma (MFH) of soft tissue. However, unlike MFH, AFX usually occurs on sun-exposed skin of the head and neck region in older adults. The diagnosis of AFX can be challenging, especially because it is often viewed as a diagnosis of exclusion after attempts are made to rule out the more clinically ominous spindle cell carcinoma and spindle cell or desmoplastic melanoma. Until recently, there have been no helpful "positive" markers for the diagnostic workup of AFX. While CD68 is usually positive in AFX, the specificity is quite low. CD163 is a monocyte/macrophage-restricted transmembrane protein that is strongly expressed on tissue macrophages. Recent reports have shown that CD163 exhibits greater tissue specificity for tumors of monocyte/macrophage lineage with no reactivity for carcinomas or melanomas. Objectives: To characterize the expression of CD163 in various cutaneous spindle cell tumors and fibrohistiocytic lesions and to determine the utility of CD163 expression in the diagnosis of AFX. Methods: 157 cutaneous spindle cell tumors and fibrohistiocytic lesions were retrospectively studied. The diagnosis of AFX was made based on histologic features and supportive immunohistochemical (IHC) findings. For most cases, a tissue microarray (TMA) was constructed from formalin-fixed paraffin-embedded tissue. Unstained slides from study cases were submitted for IHC staining with a monoclonal antibody for CD163 (clone 10D6). Cytoplasmic and membranous staining was considered positive. Staining extent was semiquantitatively classified as <10% (negative), 10% to 50%, or >50%. Intensity was graded as weak, moderate, or strong. A Fisher's exact test was used to evaluate the difference between AFX and other malignant cutaneous spindle cell tumors. Results: In normal skin, CD163 expression was observed in normal dermal dendritic cells. Among malignant cutaneous spindle cell tumors, CD163 was positive in most AFXs (11 of 14 cases; 79%), most MFHs (6 of 8 cases; 75%), a few desmoplastic melanomas (2 of 7 cases; 29%), and in a minority of leiomyosarcomas (1 of 5 cases; 20%). CD163 was negative in all spindle cell carcinomas and DFSPs. Among benign spindle cell lesions, CD163 was positive in benign fibrous histiocytoma (24 of 29 cases; 83%), fibrous papule (3 of 4 cases; 75%), and neurofibroma (1 of 10 cases; 10%). CD163 was negative in dermatomyofibroma, leiomyoma, cellular neurothekeoma, and superficial acral fibromyxoma. Most histiocytic lesions, including juvenile xanthogranuloma, were CD163-positive. Conclusions: CD163 is useful in helping to distinguish AFX from other malignant cutaneous spindle cell tumors.

Reviewer's Comments: The authors provide expression results for a broad range of cutaneous lesions. The next step should include sensitivity and specificity comparisons of CD163 with 2 recently-described "positive" AFX markers, CD99 and CD10.

Additional Keywords: None

Print Tag: Refer to original journal article
Background: Studies suggest that higher numbers of lymph nodes (LNs) retrieved from colon cancer colectomy specimens are associated with better patient survival. Based on these reports, the American College of Surgeons, the American Society for Clinical Oncology, and the National Cancer Institute recommend a minimum 12-node requirement for pathology reporting of colon cancer colectomy specimens.

Objective: To compare risk stratification of colorectal cancer using conventional lymph node N staging versus total LN retrieval, total negative LNs, total positive LNs, and the positive LN ratio.

Methods: 295 consecutive colorectal cancer patients (195 colon, 100 rectal) in Kilmarnock, Scotland, underwent curative colon/rectal resection and were evaluated for total number of LNs, adequacy of retrieval with respect to the recommended 12-node threshold, number of negative nodes, number of positive nodes, and the ratio of positive-to-total lymph nodes (pLNR). All patients were routinely monitored by CEA, liver function, and bone marker testing with follow-ups ranging from 0.1 to 6.9 years (median, 4 years). Surveillance colonoscopy and contrast-enhanced CT scan were performed at 1 and 5 years.

Results: Based on the recommended 12-node threshold, 147 of 295 pathologic evaluations produced adequate LN retrieval (49.8%), but there was no negative impact on survival associated with inadequate LN retrieval for any TNM stage of disease (P >0.050). By univariate analysis, there was no impact on survival in either colon or rectal cancer related to total number of retrieved LNs or total number of negative LNs. By univariate analysis in both colon and rectal cancer, N-stage, pLNR, and total number of positive LNs were all prognostically significant. However, by multivariate analysis, only pLNR and patient age independently predicted survival for both colon and rectal cancer patients. Four increasing levels of pLNRs defined at <0.05, 0.05 to 0.19, 0.20 to 0.39, and 0.40 to 1.0 effectively stratified four progressively worse survival groups (P <0.001). Applying pLNRs within the N1 and N2 subgroups further substratified both of these into distinct survival groups.

Conclusions: The relative ratio of positive lymph nodes identified in cancerous colorectal resections is prognostically superior to traditional N-staging and may diminish the perceived need for rigid thresholds for total lymph node retrieval.

Reviewer's Comments: Surgeons and pathologists should make every effort to maximize the number of LNs retrieved from cancerous colorectal resections. However, a rigid threshold for total LN retrieval (e.g. 12-node minimum) does not account for variables that impact on how many nodes can be identified, such as neoadjuvant therapy, use of fat clearing solutions, left-sided versus right-sided resections, or age and immunologic status of the patient.

Additional Keywords: None

Print Tag: Refer to original journal article
Some Breast ALCL Cases Strongly Associated With Implants

**Anaplastic Large Cell Lymphoma Involving the Breast: A Clinicopathologic Study of 6 Cases and Review of the Literature.**
Miranda RN, Lin L, et al::
Arch Pathol Lab Med 2009; 133 (September): 1383-1390

Anaplastic large cell lymphoma is a rare breast malignancy that has a strong relationship with breast implants.

**Background:** The most common nonepithelial neoplasm of the breast is hematological, but most primary lymphomas of the breast are of B-cell lineage. However, rare cases of anaplastic large cell lymphoma (ALCL) have been reported, notably having a strong association with breast implants.

**Objective:** To characterize the clinicopathologic features of a series of ALCL occurring in the breast.

**Methods:** A review of archived files dating to 1986 identified 6 cases fulfilling the World Health Organization’s criteria for ALCL. Immunohistochemical stains for CD3, CD20, CD45, CD45RO, ALK1, CD43, and CD30 were performed. Gene rearrangement studies for T-cell receptor γ-chain were performed on formalin-fixed paraffin-embedded tissue using PCR techniques.

**Results:** All 6 patients were women presenting with a unilateral palpable breast mass subjected to excisional biopsy (n=3), needle-core biopsy (n=2), and capsulectomy (n=1). The median age at diagnosis was 52 years, and, of the data available, most women presented with normal peripheral blood counts, except one with mild anemia and another with mild thrombocytosis. Four of the patients had ALK-negative tumors, of which 2 had previously been diagnosed with cutaneous ALCL. One of these 2 women had ALCL associated with breast implants inserted 8 years before diagnosis. Both were positive for T-cell gene rearrangements. The other 2 patients with ALK-negative ALCL did not have a history of cutaneous ALCL, and both were associated with breast implants. One had a history of treatment for classic nodular sclerosis Hodgkin lymphoma, which in retrospect may represent axillary lymph node involvement by the ALK-negative ALCL. The 2 patients with ALK-positive ALCL had breast involvement in clinical stage IV disease, and neither of these cases was associated with breast implants. Of the 3 cases associated with an implant, 2 had a seroma-like fluid collection. Follow-up was available for 5 of 6 patients after undergoing various chemotherapeutic regimes with or without radiation therapy. Only 1 patient died of disease, which occurred 1.5 years after diagnosis of stage IV ALK-positive ALCL. The other 4 cases with adequate follow-up were alive at a mean of 4.1 years after diagnosis. By definition, all cases consisted of large anaplastic CD30-positive cells that also expressed T-cell markers.

**Conclusions:** ALCL rarely involves the breast, but it may develop in the association with cutaneous ALK-negative ALCL, breast implants (described as a fibrous capsule with or without a seroma), or systemic ALK-positive ALCL.

**Reviewer's Comments:** The authors confirm previous reports of a strong association with breast implants and ALCL. They also describe ALK-positive ALCL involving the breast in widespread systemic disease.

Additional Keywords: None

Print Tag: Refer to original journal article
Background: Gastrointestinal stromal tumors (GISTs) occur throughout the GI tract and abdomen. The tumors usually have mutations of either the \textit{KIT} or \textit{PDGFRA} genes. Identification of these tumors and distinction from other mesenchymal tumors is important, especially as inhibitors to the involved tyrosine kinases have been developed. Helpful immunohistochemistry for the diagnosis of GISTs include antibodies to CD34 and KIT. Recently, polyclonal and monoclonal antibodies have been developed to DOG1, a protein overexpressed in GISTs.

Objective: To review the expression of DOG1 in a large series of GISTs and to compare these results with those of KIT immunostaining. Entities included in the differential diagnosis for GISTs were also studied.

Methods: Nearly 40 years of material was available from the archives of a single institution. Tissue arrays were available that harbored 1168 GISTs and 672 other tumors. Immunostaining was performed with antibodies to DOG1 and KIT. Results were compared for tumor type, site of tumor, and phenotype of tumor. Data were gathered regarding molecular changes in tumors noted to be immunonegative with KIT and DOG1 antibodies.

Results: Overall, 94\% and 95\% of GISTs were immunoreactive with antibodies to DOG1 and KIT, respectively. Of the GISTs studied, 92\% were immunoreactive with both antibodies, and 3\% were immunonegative for both antibodies. Tumors stained relatively similar regardless of location or phenotype. However, gastric epithelioid GISTs were slightly more likely to stain with antibodies to DOG1, whereas small intestinal GISTs were slightly more likely to stain with antibodies to KIT. Eight of 12 cases that were found to be nonreactive to both KIT and DOG1 had wild-type \textit{KIT} and \textit{PDGFRA} genes. Fifteen of 562 non-GIST mesenchymal tumors were immunoreactive with antibodies to DOG1, including 5 uterine-type leiomyomas, 4 cases of leiomyomatosis, and 6 synovial sarcomas. Twenty-two of 110 epithelial tumors were immunoreactive with antibodies to DOG1, including 11 gastric adenocarcinomas and 9 esophageal squamous cell carcinomas.

Conclusions: DOG1 is helpful for the diagnosis of GISTs and its distinction from other tumors included in the differential diagnosis.

Reviewer's Comments: This paper includes a huge series of GISTs that underwent DOG1 immunostaining. The antibody may help identify 2\% to 3\% more GISTs when used in combination with KIT staining compared to KIT staining alone.
Perinephric atypical (myo)fibroblastic proliferations adjacent to a renal cell carcinoma may be misdiagnosed as well-differentiated liposarcoma.

**Background:** Sarcomatoid foci of renal cell carcinoma (RCC) confer a poor prognosis and can be associated with any of the histologic subtypes: clear cell, papillary, chromophobe, or collecting duct. This metaplastic component is typically anaplastic spindle cells with or without heterologous differentiation. Liposarcomas also occur in the retroperitoneum, especially the sclerosing form, which displays variable adipocytes surrounded by fibrous septa containing atypical/hyperchromatic spindle cells. FISH for *MDM2* gene has been developed as a marker for amplification, commonly demonstrated in well-differentiated liposarcomas (WDL). The observation of markedly atypical cells in the perinephric fat adjacent to RCC in 2 separate consultation cases were submitted with the question of an incidental WDL.

**Objective:** To characterize foci of atypical spindle cells in the perinephric fat of surgical specimens for RCC.

**Methods:** In addition to the 2 consultation cases submitted for review of the atypical spindle cell proliferation, 59 consecutive nephrectomy specimens diagnosed as RCC during the 14-month study interval were reviewed for similar lesions. Immunohistochemistry for AE1/AE3, CAM 5.2, cytokeratin 7, EMA, S100, SMA, desmin, and Melan A were performed and interpreted with a semiquantitative score. FISH for amplification of the *MDM2* gene was performed in 11 cases on paraffin-embedded tissue sections.

**Results:** Of the 59 nephrectomy cases reviewed, 12 (20%) contained a focus of atypical spindle cell proliferation in the perinephric fat. Of the 10 cases that had invaded through the renal capsule into the perinephric fat, 3 (30%) contained an adjacent atypical spindle cell proliferation. Of the remaining 49 cases that did not have capsular penetration, 9 (18%) contained an adjacent atypical spindle cell proliferation. Of the 14 lesions, all contained haphazardly arranged, enlarged, and hyperchromatic spindle cells embedded in a myxoid and collagenous stroma with some fibrous septae. Some contained areas having epithelioid cells with eccentric nuclei, while 3 cases even had "floret-like" multinucleated cells. Mitotic figures, necrosis, and lipoblasts were absent. All cases were negative for cytokeratins, EMA, S-100, HMB-45, and Melan A. Focal to variably positive reactions were noted with SMA and desmin in a few cases. FISH was negative for MDM2 amplification in all 11 cases examined.

**Conclusions:** Even in the absence of capsular penetration, RCC can be associated with a perinephric pseudosarcomatous fibroblastic/myofibroblastic proliferation that mimics a WDL.

**Reviewer's Comments:** The authors considered a sarcomatoid component of RCC or collision tumors, such as angiomyolipoma or liposarcoma, but immunohistochemistry and FISH contributed to their exclusion. Only focal myofibroblastic differentiation was observed, supporting their interpretation of a pseudosarcomatous proliferation.

Additional Keywords: None

Print Tag: Refer to original journal article
A subset of renal oncocytomas has overexpression of cyclin D1 and CCND1 rearrangement, but chromophobe renal cell carcinomas do not show either of these changes.

**Background:** Renal oncocytoma (RO) is a benign tumor of the kidney which can be difficult to distinguish from chromophobe renal cell carcinoma (ChRCC). Recent studies have demonstrated a subset of ROs that overexpress cyclin D1, and some also show chromosomal rearrangements at 11q13, involving the CCND1 locus.

**Objective:** To evaluate cyclin D1 overexpression and CCND1 rearrangement in a large series of ROs and ChRCCs with regard to clinical and histologic features.

**Methods:** Formalin-fixed paraffin-embedded tissue from 63 cases of primary RO and 36 cases of ChRCC were evaluated. Each case was stained with antibodies to cyclin D1, and only nuclear staining was considered positive. Fluorescent in-situ hybridization (FISH) with a dual-color break-apart probe was used to detect the presence or absence of rearrangement of the CCND1 gene region. A normal cut-off value of 4% of cells with an abnormal signal pattern was established using normal kidney specimens; tumors were considered positive for CCND1 rearrangement if >4% of the 200 cells analyzed demonstrated separation of the FISH probe.

**Results:** All cases of ChRCC were negative for both cyclin D1 staining and CCND1 rearrangement by FISH. Of the 63 RO cases, 21 (33%) overexpressed by cyclin D1 (intense nuclear staining in >90% of tumor cells). Of the 21 RO cases with cyclinD1 overexpression, 13 (57%) showed CCND1 rearrangement by FISH. One additional case of RO, which was negative for cyclin D1, also showed CCND1 rearrangement. There was no histologic difference between ROs with and without cyclin D1 overexpression or CCND1 rearrangement. There was an association of multiple ROs (either synchronous or metachronous) with absence of cyclin D1 overexpression or CCND1 rearrangement (16 of 42 cases). In comparison, only 1 of 21 cases with cyclin D1 overexpression had synchronous multiple oncocytomas. **Conclusion:** A subset of renal oncocytomas has overexpression of cyclin D1, and approximately 50% of these also show CCND1 rearrangement. These changes are absent in chromophobe renal cell carcinomas, and a positive result may be helpful in ruling out a diagnosis of ChRCC. In addition, there is a negative association of multiple ROs and cyclin D1 expression; this suggests an alternate pathway of tumorigenesis in a subset of ROs.

**Reviewer’s Comments:** Cyclin D1 overexpression by immunohistochemistry appears to be fairly specific but poorly sensitive for a diagnosis of RO. This may have the greatest utility when used as part of a panel of markers or on small biopsies.

Additional Keywords: None

Print Tag: Refer to original journal article
In patients with suspected acute myocardial infarction, saliva can be used to accurately measure a variety of cardiac biomarkers to assess for myocyte damage.

**Background:** Although ECG testing represents the most common initial test performed en route to hospitals in patients with chest pain, a significant number of patients only have confirmed acute myocardial infarction (AMI) after testing with additional cardiac biomarkers. While point-of-care testing programs and other efficiency measures have improved the time it takes to receive results of serum cardiac biomarker testing, the results of some studies suggest that it still takes up to 1 hour for 25% of such results to become available. Recent interest in the use of oral fluid samples to diagnose disease has confirmed that saliva can be used to measure enzymes, antibodies, microbes, and RNA molecules, among other substances. **Objectives:** To determine if serum biomarkers used to diagnose AMI can be reliably measured in unstimulated whole saliva, and to determine if a nano-biochip testing methodology might be feasible for rapidly screening patients for AMI.

**Design:** A cross-sectional clinical case-control study.

**Participants:** 41 test subjects and 43 apparently healthy age- and gender-matched control subjects. The test subjects were recruited within 48 hours of the onset of AMI symptoms.

**Methods:** Subjects with presumptive diagnoses of ST elevation MI (STEMI) had ST-segment elevation on ECG and increased cardiac biomarkers. Subjects with diagnoses of non-ST-segment elevation MI (NSTEMI) showed ECG abnormalities other than classic ST-segment elevation, followed by confirmatory positive cardiac troponin I (cTnI) testing. Serum and unstimulated saliva samples were collected and analyzed in duplicate for cardiac enzymes as well as a panel of 21 biomarkers. Standard cardiac enzyme markers included BNP, MYO, CK-MB, and cTnI. The additional panel of 21 biomarkers included CRP, IL-6, MPO, TNF-α, IL-18, E-selectin, and MMP-9, among others. Differences between median biomarker concentrations in saliva were determined for AMI versus non-AMI subjects. Logistic regression and ROC analysis were also performed to evaluate the diagnostic utility of each individual biomarker or biomarker combinations in screening for AMI. The tests on unstimulated whole saliva were adapted for use using a novel lab-on-a-chip platform.

**Results:** Significant differences in concentrations were detected between the standard and novel biomarkers of subjects with and without AMI. A saliva-based biomarker panel utilizing myeloperoxidase, myoglobin, and C-reactive protein showed statistically significant diagnostic power (area under the curve [AUC], 0.85; \( P <0.0001 \)). This was further enhanced when these results were interpreted in conjunction with ECG results (AUC=0.96). After confirming the presence of significant differences, a testing panel for whole saliva was successfully implemented using lab-on-a-chip technology. **Conclusions:** Screening assays of various biomarkers in whole saliva using lab-on-a-chip technology may provide more rapid diagnosis of AMI when performed along with traditional ECG testing.

**Reviewer’s Comments:** Lab-on-a-chip technology has emerged as a compelling platform for multiplex testing of a wide range of important analytes in serum and saliva.

Additional Keywords: None

Print Tag: Refer to original journal article
Breast Cancer DFS Worsens With Micrometastases, Isolated Tumor Cells

Micrometastases or Isolated Tumor Cells and the Outcome of Breast Cancer.
de Boer M, van Deurzen CH, et al::

Female breast cancer patients with isolated tumor cells or micrometastases in regional lymph nodes have significantly lower 5-year disease-free survival rates that can be improved by receiving systemic adjuvant therapy.

**Background:** In breast cancer patients, the Cancer Staging Manual published by the American Joint Committee on Cancer arbitrarily distinguishes nodal isolated tumor cells (≤0.2 mm) from micrometastases, which are >0.2 mm and ≤2.0 mm. The prognostic significance of breast cancer isolated tumor cells and micrometastases detected during the sentinel node biopsy era remains controversial.

**Objective:** To determine the prognostic significance of isolated tumor cells or micrometastases in lymph nodes from female breast cancer patients with otherwise favorable pathologic features.

**Design:** Retrospective review.

**Participants:** 2707 patients in the Netherlands Cancer Registry with invasive breast cancer, all of whom had sentinel-node biopsies and favorable tumor features (any histologic grade ≤1 cm size, or grade 1 or 2 tumors <3 cm) including 856 node-negative with no systemic adjuvant therapy, 856 with isolated tumor cells or micrometastases with no systemic adjuvant therapy, and 995 with isolated tumor cells or micrometastases with systemic adjuvant therapy. The median follow-up was 5.1 years.

**Results:** The 5-year disease-free survival (DFS) rate among patients who did not receive adjuvant therapy was 77.2% in patients with isolated tumor cells, 75.9% in those with micrometastases, and 85.7% in those with node-negative disease (P<0.001). Even after controlling for other adverse indicators (age, tumor size, grade, hormone receptor status), both of the node-positive, no-adjuvant-therapy groups had significantly higher adverse risks than did the node-negative, no-adjuvant therapy group. Whether or not patients had axillary lymph node dissection did not alter the risk. Among patients who received adjuvant therapy, the 5-year DFS rate was 83.0% in those with isolated tumor cells and 87.9% in those with micrometastases. The DFS rate was improved with adjuvant therapy as compared to node-positive, no-adjuvant-therapy patients. The improved DFS rate in node-positive patients who received adjuvant therapy remained significant even after controlling for other adverse indicators.

**Conclusions:** Female breast cancer patients with isolated tumor cells or micrometastases in regional lymph nodes have significantly lower 5-year DFS rates that can be improved by receiving systemic adjuvant therapy.

**Reviewer’s Comments:** These results are based on combined sentinel node and regional node status (when axillary dissection was performed) as opposed to sentinel node-only status. Of note, DFS rates were very similar between patients with isolated tumor cells and patients with micrometastases, suggesting that the volume of metastatic disease did not have a large clinical impact and calling into question the classification of isolated tumor cells as node-negative (pN0[i+]).

Additional Keywords: None

Print Tag: Refer to original journal article
Thread-Like Bridging Strands Help Identify Adenomatoid Tumors

Adenomatoid Tumors of the Female and Male Genital Tracts: A Clinicopathological and Immunohistochemical Study of 44 Cases.
Sangoi AR, McKenney JK, et al.; Mod Pathol 2009; 22 (September): 1228-1235

Adenomatoid tumors consistently stain for pankeratin, calretinin, WT-1, and D2-40. Thread-like bridging strands are consistently present in all genital tract adenomatoid tumors.

Background: Adenomatoid tumors are classically defined as benign mesotheliomas commonly observed in both the male and female genital tract. While classic mesothelial markers are known to be positive in such lesions, newer markers have not been studied. Objective: To compare the histologic and immunohistochemical characteristics of adenomatoid tumors from female and male genital tracts. Methods: 44 adenomatoid tumors were retrieved from the archived files and were reviewed by 3 pathologists for a consensus of diagnosis based on H&E staining. Immunohistochemistry (IHC) was performed and scored semiquantitatively for pankeratin, CK 5/6, calretinin, D2-40, WT-1 and caldesmon. Results: Within the female tract, 26 of 32 tumors were myometrial (81%), 4 were in the fallopian tube, and 2 were in the ovary. In the male tract, 8 were epididymal (75%), and 4 were in the paratestis. Most lesions were incidental in women, while men presented with a mass lesion. None recurred or progressed during a mean follow-up of 95.5 months. All tumors demonstrated thin bridging strands across tubular spaces, and nearly all had signet-ring cells. The lesions contained lymphoid aggregates in all males but in only a few females. Infarction was noted in small patient subsets, and it sometimes contained areas of hyalinized stroma and/or a myofibroblastic proliferation. An infiltrative growth pattern was noted in 75% of the cases overall, but none invaded an adjacent organ or demonstrated marked cytologic atypia, tumor-type necrosis, or mitotic activity. The fallopian tube lesions were notably circumscribed. Nearly all lesions stained positively for pankeratin, calretinin, D2-40, and WT-1. However intense background staining of calretinin and WT-1 in the female genital tract made interpretation of tumor cells more difficult. CK5/6 and caldesmon stained only a minor percentage of cases.

Conclusions: Adenomatoid tumors commonly have an infiltrative growth with a thin bridging strand pattern and signet-ring cells which stain for pankeratin, calretinin, WT-1 and D2-40, the last of which is easiest to interpret in lesions of the female genital tract. While common in tumors of the male genital tract, lymphoid aggregates may be lacking in females.

Reviewer’s Comments: Knowledge of this IHC profile will be useful in evaluating adenomatoid tumors that fall outside the genital tract, where the differential diagnosis may be expanded considerably to include vascular lesions, signet-ring carcinomas, perivascular epithelioid cell tumors (PEComas), and others.

Additional Keywords: None

Print Tag: Refer to original journal article
Expect Aggressive Behavior in SFTs With Dedifferentiation

Expanding the Spectrum of Malignant Progression in Solitary Fibrous Tumors: A Study of 8 Cases With a Discrete Anaplastic Component—Is This Dedifferentiated SFT?

Mosquera JM, Fletcher CD::

Rare solitary fibrous tumors have foci of dedifferentiation, which poses a serious risk for poor behaviour and is associated with distinct changes in protein expression by the tumor.

**Background:** A number of soft tissue tumors have been described that undergo dedifferentiation, including liposarcomas, chondrosarcomas, osteosarcomas and chordomas. This is generally described as an abrupt change from a well-differentiated component to a high-grade undifferentiated component of the same tumor. P53, a tumor suppressor gene, is frequently mutated in the dedifferentiated components of these tumors, usually demonstrated by overexpression of the protein by immunohistochemistry (IHC). This has been shown with a number of different salivary gland tumors and sarcomas. P16 has also been shown to be overexpressed in the high-grade components of these tumors.

**Objective:** To describe the characteristics and immunohistochemical features of a series of solitary fibrous tumors (SFTs) shown to undergo dedifferentiation.

**Methods:** A single individual's consultation files were used to collect 8 examples of dedifferentiated SFTs, which was defined as abrupt transition between a morphologically benign-appearing SFT (no atypia, hypercellularity, or significant mitotic activity) to a high-grade component that had no resemblance to typical SFT. IHC was performed with antibodies to CD34, CD99, S100, desmin, pankeratin, AE1/AE3, EMA, p53, and p16. Clinical and follow-up information were obtained.

**Results:** This study included 3 men and 5 women who presented between the ages of 40 and 76 years. Two cases were intrathoracic, and the remaining cases occurred throughout the body. Eight patients initially underwent surgical excision alone, and 2 had positive margins. Four patients subsequently received radiation therapy, chemotherapy, or a combination of both. Of the 7 patients with follow-up, 3 died of disease, 1 was alive with disease, and 3 were alive with no evidence of disease. Tumors ranged in size from 3.4 to 20 cm. Most tumors showed gross evidence of necrosis. The dedifferentiated components of the tumors showed sheet-like growth of epithelioid or round cells, sometimes with spindled cells. Cystic degeneration and necrosis were frequently present. Mitotic rates varied from 3 to 25 mitotic figures per 10 high-powered fields. Within these components, there were no features suggestive of SFT. Immunoreactivity for both CD34 and CD99 was frequently lost in the dedifferentiated areas. Rare immunoreactivity to keratin and EMA was seen, and tumors were universally nonreactive with antibodies to S100 and desmin. Both p53 and p16 were usually overexpressed in the dedifferentiated areas.

**Conclusions:** Dedifferentiation with SFTs poses a serious risk for poor behavior. This change is associated with distinct changes in protein expression by the tumor.

**Reviewer's Comments:** Pathologists can now add SFT to their list of tumors that can undergo dedifferentiation. They should be aware of this when they review sarcomas that have an otherwise nondescript high-grade morphology.

Additional Keywords: None

Print Tag: Refer to original journal article
Intercalated duct lesions of the salivary glands are uncommon and show at least 2 growth patterns (hyperplasia and adenoma). These lesions are sometimes associated with basal cell neoplasms of the salivary gland.

**Background:** Intercalated duct lesions (IDLs) of the salivary glands are uncommon lesions, and their relationship to salivary gland neoplasia is unclear. Some have reported the lesions to be seen with epithelial-myoeptihelial carcinomas and basal cell neoplasms, whereas others have reported IDLs to be associated with chronic sialadenitis and other types of neoplasms. While many IDLs are believed to represent hyperplastic lesions, some studies have described cases that could be considered to be adenomas.

**Objective:** To review a series of IDLs seen at multiple institutions.

**Methods:** The surgical pathology files of 3 institutions were reviewed for all cases of IDLs, and 34 cases from 32 different patients were identified. Lesions were considered to be IDLs when >1 mm in size and composed of a proliferation of ducts resembling normal intercalated ducts with or without acinic or mucinous cells. Cases with atrophy, chronic inflammation, and significant stroma were excluded. Immunohistochemistry was performed with a number of different antibodies.

**Results:** Nearly two-thirds of patients were women (age range, 19 to 80 years). Most IDLs were in the parotid gland, and 6% were located in the oral cavity. Most lesions were <8 mm in size and were unifocal. Approximately two-thirds of cases were considered hyperplastic and were composed of nonencapsulated proliferations that blended imperceptibly into the surrounding salivary glands. Ductal cells were uniform and cuboidal, and they lacked pleomorphism and mitotic activity. Myoepithelial cells were difficult to discern. Nearly one-third of cases were considered adenomas. These were discrete, rounded, and at least partially encapsulated. Otherwise, they resembled the cases of hyperplasia. Four cases showed features of both hyperplasia and adenoma. Other salivary gland tumors were identified in almost 60% of cases, including 8 basal cell adenomas, 2 basal cell adenocarcinomas, 2 pleomorphic adenomas, 2 mucoepidermoid carcinomas, 1 Warthin tumor, and 1 acinic cell carcinoma. Lysozyme and CK7 immunostaining was seen with all ductal cells. Focal or diffuse estrogen receptor staining was seen in all cases. Calponin and CK14 demonstrated a thin myoepithelial layer around all ducts. Three-quarters of the cases showed luminal immunoreactivity with antibodies to S100.

**Conclusions:** Intercalated duct lesions are uncommon and show at least 2 growth patterns. They are sometimes associated with basal cell adenomas and other salivary gland tumors.

**Reviewer’s Comments:** It is unclear if IDLs represent true neoplasms, precursors to other neoplasms, or reactive hyperplasias. Clonality testing may help shed some light on these peculiar lesions.

Additional Keywords: None

Print Tag: Refer to original journal article
Myxoid liposarcomas appear to have an immature adipocytic phenotype (high levels of PPARγ; low levels of CEBPα). Novel negative prognostic markers of outcome include RET, IGF1R, and IGF2.

**Background:** Myxoid liposarcomas (MLs) have a characteristic chromosomal translocation, t(12;16) (FUS-DDIT3). Although in vitro studies suggest that FUS-DDIT3 acts as an oncogene by blocking adipocytic differentiation and by upregulating the cell cycle, there is limited clinical data to support this. Expression profiling has revealed a distinctive immature adipocytic signature in some ML tumor samples.

**Objective:** To assess the protein expression of key adipogenic regulatory molecules using tissue microarray (TMA) immunohistochemistry (IHC) in benign and malignant fatty tissues, along with potential prognostic biomarkers.

**Methods:** 32 primary MLs, 9 well-differentiated liposarcomas, 6 pleomorphic liposarcomas, 10 lipomas, and 9 samples of normal fat were used to build a lipogenic TMA using 3 formalin-fixed paraffin embedded cores per case. IHC for key adipogenic regulatory proteins (PPARγ, DLK1, CEBPα, 11βHSD2, HPGD) and cell cycle proliferation proteins (RET, IGF1R, IGF2, Ki67) was performed on TMA sections. IHC staining results were semiquantitatively assessed, and the 3 core values were averaged.

**Results:** All MLs showed at least some PPARγ staining, while only 13 of 34 other specimens were positive at low levels (100% sensitivity, 62% specificity). Most MLs were negative for CEBPα and DLK1, and showed moderate to high HPGD and 11βHSD2 expression. In comparison, normal fat and lipomas showed low expression of all markers, and well-differentiated liposarcoma had increased levels of CEBPα. Pleomorphic liposarcomas showed increased levels of DLK1 and 11βHSD2, but were negative for PPARγ, HPGD, and CEBPα. Ki-67 levels were much higher in MLs than in normal fat, and the highest levels of RET were observed in pleomorphic liposarcomas. On univariate analysis, high levels of RET, IGF2, and IGF1R correlated with significantly worse patient prognosis in patients with MLs. Multivariate analysis could not be performed due to the small patient cohort. **Conclusion:** Myxoid liposarcomas appear to have an immature adipocytic phenotype, as demonstrated by high levels of PPARγ and low levels of CEBPα. In patients with MLs, novel negative prognostic markers of outcome include RET, IGF1R, and IGF2.

**Reviewer’s Comments:** Additional studies with larger patient populations and outcome data are needed to assess the potential prognostic value of RET, IGF1R and IGF2 in patients with MLs.

Additional Keywords: None

Print Tag: Refer to original journal article
An immunohistochemical panel of cancer-related proteins may be useful for distinguishing multiple primary lung cancers from intrapulmonary metastasis, thus helping with selection of the appropriate therapy.

**Background:** Multiple primary lung cancers (MPLCs) have been reported to occur in as many as 15% of patients with lung cancer. Distinguishing a synchronous/metachronous primary lung cancer from an intrapulmonary metastasis (IPM) can be difficult. Importantly, the overall survival of patients with IPM is significantly worse. Several authors have used gene-mutational analysis, but this is time-consuming and expensive.

**Objective:** To evaluate the ability of immunohistochemistry (IHC) to differentiate between MPLCs and IPMs.

**Participants:** 50 patients with MPLCs (diagnosed according to Martini and Melamed's criteria), 20 patients with lung cancer and IPM, and 30 patients with lung cancer with lymph node metastasis.

**Methods:** In all patients with MPLCs, the tumors were of the same histologic subtype (most commonly adenocarcinoma). Each case was stained with antibodies to p53, p16, p27, and c-erbB2 on both the primary tumor and a lymph node metastasis, intrapulmonary metastasis, or second primary tumor. The percentage of immunoreactive tumor cells was estimated on each tumor part. The percent difference in IHC staining between part types was calculated, and the sum of these differences was recorded. A positive reaction was defined as nuclear staining for p53, p16, and p27 and as cell membrane staining with c-erbB2.

**Results:** When compared with the primary tumor, the sum difference in IHC staining for lymph node metastases ranged from 30% to 90% (mean, 62%). Therefore, a difference of >90% between two tumors was chosen as the criterion for identifying MPLCs. Using this criterion, 41 of 50 patients (82%) clinically diagnosed as having MPLCs had a sum difference of >90% (confirmed separate primaries), and 9 patients were reclassified as having IPMs. Of 20 patients clinically diagnosed with IPMs, 16 (80%) had a sum difference of ≤90% (confirmed IPM), and 4 patients were reclassified as having MPLCs. The 5-year survival rate for patients initially classified as having MPLCs and IPMs was 62% and 46%, respectively. When reclassified according to the IHC comparison results, the 5-year survival of patients with MPLCs and IPMs was 81% and 40%, respectively.

**Conclusions:** An IHC profile of cancer-related proteins can be a useful tool to distinguish MPLCs from IPMs. This has a large impact on tumor staging and the overall prediction of survival.

**Reviewer’s Comments:** The poor prognosis of patients with IPM is reflected in its staging as T4 if within the same lobe and as M1 if in other lobes of the lung. In situations where confirmation of the tumor stage is critical, IHC staining shows promise in identifying separate primaries.

Additional Keywords: None

Print Tag: Refer to original journal article
Core needle biopsies are highly accurate at diagnosing invasive cancer of the breast, but they are less accurate for the diagnosis of ductal carcinoma in situ.

**Background:** For preoperative assessment of women with suspected breast cancer, core needle biopsy is considered to be highly accurate. Results of core biopsy often influence the extent of surgery and the decision to initiate neoadjuvant systemic therapy, based on the presence of ductal carcinoma in situ (DCIS) and the results of hormone receptor studies and Her2/neu status, respectively. Core biopsy, however, is subject to sampling error and may not represent all histological features seen in the larger excisional specimens.

**Objective:** To compare the histologic features (tumor type and grade), the presence or absence of intraductal lesions, and the hormone receptor and Her2/neu status of the tumor in core biopsies compared to surgical excision specimens.

**Methods:** During a 1-year study interval at a large university hospital, 567 consecutive core biopsies had subsequent surgical excisional procedures performed. Biopsies were either taken using automated ultrasound-assisted biopsy (14-gauge needle) or with a stereotactic vacuum-assisted biopsy (11-gauge needle). The core biopsy and subsequent surgical excision specimens were routinely processed. All tissues were evaluated by 2 pathologists in a similar fashion, using the current WHO criteria for noninvasive and invasive breast cancer. Invasive cancers were graded using the Elston and Ellis criteria, while DCIS was graded as low, intermediate, or high based on criteria of the World Health Organization. For all cases with sufficient tissue, immunohistochemical (IHC) assessment of estrogen receptor (ER), progesterone receptor (PR), and Her2/neu status was performed. Fluorescence in situ hybridization (FISH) analysis for Her2/neu status was performed instead of IHC in a subset of cases. Percentage agreement and kappa values (if possible) were determined for each of the following parameters: histological type, histological grade, intraductal component, hormone receptor status, and Her2/neu status. **Results:** In 99.6% of cases diagnosed as invasive carcinoma on core biopsy, the excisional specimen showed identical results. However, DCIS was found in 32% of surgical excision cases for which no evidence of DCIS was seen on core biopsy. There was a high rate of agreement among results for ER and PR status, although only 54% of cases showed complete concordance for Her2/neu analysis. **Conclusions:** Core needle biopsy is highly accurate for the diagnosis of invasive carcinoma. Biopsy is less accurate, however, for the diagnosis of DCIS.

**Reviewer’s Comments:** As the authors point out in their discussion, the detection of DCIS on core needle biopsy has important treatment implications. Increasing the number of cores in combination with careful clinical and radiologic correlation may improve the needle biopsy detection rate of DCIS.
The DLC1 gene has a tumor suppressor function. The mechanism may include regulation of cyclinD1 and p21.

**Background:** DLC1 (deleted in liver cancer-1) is a relatively novel candidate tumor suppressor gene that was first described in primary hepatocellular carcinoma. Since this initial discovery, numerous investigators have demonstrated that the DLC1 protein is inactive in a variety of human cancers, including colorectal carcinoma.

**Objective:** To study the function of DLC1 and to elucidate its role in colon carcinogenesis. **Methods:** The authors constructed a pcDNA3.1 vector containing the DLC1 gene and then transfected this vector into a known colon cancer cell line called HT29, which lacked expression of DLC1. Transfection assays, RNA extraction and RT-PCR, Western blot test, colony formation assays, and flow cytometric analysis were performed. **Results:** The restoration of DLC1 expression in HT29 cells through the transfection experiment significantly inhibited the migration and proliferation activity of the transfected cells. Flow cytometry analysis clearly demonstrated that apoptosis was induced by DLC1 transfection into the HT29 cells. Arrest of the cell cycle in S-phase was also observed. Compared to the control wild-type HT29 cells, there was down-regulation of both cyclinD1 mRNA and protein expression in the pcDNA3.1-DLC1-HT29 cells. In contrast, there was an increase in p21 expression in the pcDNA3.1-DLC1-HT29 cells as compared to the wild-type cells. **Conclusions:** The authors provide supporting evidence that the DLC1 gene has a tumor suppressor function. The mechanism may include regulation of cyclinD1 and p21. Thus, the DLC1 gene may provide a model therapy for the inhibition of colorectal carcinoma.

**Reviewer’s Comments:** The confirmation of the tumor suppressor role of DLC1 is an important finding that will certainly be applicable to understanding its role in other solid malignancies.

Additional Keywords: None

Print Tag: Refer to original journal article
The morphologic spectrum of nodal marginal zone lymphoma includes diffuse, nodular/follicular, interfollicular, and perifollicular growth.

**Background:** Nodal marginal zone lymphoma (NMZL) is relatively rare and remains clinically distinguished from extranodal (mucosal) and splenic subtypes of marginal zone lymphomas. Diagnostic challenges include lack of a specific immunophenotype, variable histomorphology (including follicular colonization), and the general rarity of NMZL.

**Objective:** To characterize the morphologic spectrum of NMZL.

**Methods:** 51 cases of NMZL were identified in the pathology files at Stanford University and were retrospectively evaluated. Cases with extranodal, splenic, or splenic hilar lymph node involvement were excluded. Also, cases with >50% large cells or sheets of large cells were excluded.

**Results:** Architectural growth patterns included (1) "diffuse" nodal effacement (75% of cases), although roughly half of these had minor areas with vaguely nodular growth; (2) densely packed "nodular/follicular" zones (10% of cases) that were distinct from benign interfollicular zones; (3) malignant "interfollicular" zones (14% of cases) surrounding preserved germinal centers; and (4) "perifollicular" growth (a single case) with rings of neoplastic cells surrounding benign follicles. The inverse follicular pattern often seen in splenic marginal zone lymphoma was not seen by routine H&E staining. Most cases (75%) contained a mixture of small and large neoplastic cells, most often <20% and always <50% of cells. Monocytoid cells were identified in 71%, and increased plasma cells were seen in 47%. Tumor cell staining for bcl-2 was present in 43%, and staining for CD43 was present in 23%. Plasma cell clonality was demonstrated in 31%. An inverted pattern of bcl-2 staining was distinct from the follicular pattern seen in lymphoma. All cases were negative for follicular cell markers, CD10, bcl-6, HGAL, and LMO2. Staining for the follicular dendritic cell (FDC) marker CD21 highlighted disrupted FDC networks in 71%, with follicle colonization and aberrant intrafollicular FDC networks in 27%.

**Conclusions:** The morphologic and immunophenotypic spectrum of nodal marginal zone lymphoma as well as its relation to markers of follicular cell and dendritic cell differentiation are described.

**Reviewer's Comments:** The distinction of NMZL from lymphoplasmacytic lymphoma remains ill-defined. Cases with predominant monoclonal plasma cells, predominant marrow involvement, and other features of lymphoplasmacytic lymphoma were excluded from this series.

Additional Keywords: None

Print Tag: Refer to original journal article
Copper Deficiency Often Overlooked in Cytopenia Cases

Hematogone Hyperplasia in Copper Deficiency.
Sutton L, Vusirikala M:

In patients presenting with peripheral blood cytopenias, increased bone marrow hematogones accompanied by immature granulocyte and erythroid vacuolization should prompt clinical evaluation for copper deficiency.

Background: In the absence of obvious bone marrow dysplasia, determining the cause of peripheral blood cytopenias can be challenging. Mild morphologic features of dys hematopoesis can be associated with benign reactive changes secondary to nutritional deficiencies, infections, autoimmune or paraneoplastic syndromes, chemotherapy, or drug-related effects. Instead of mistakenly diagnosing such cases as myelodysplastic syndromes (MDS), which have a roughly 30% likelihood of responding to therapy, it is important to recognize benign treatable causes of peripheral blood cytopenias.

Objective: To distinguish clinicopathologic features of copper deficiency from MDS in patients with peripheral cytopenias.

Methods: During evaluation for lower extremity neuropathy at the University of Texas Southwestern Medical Center at Dallas, 2 adult males (ages 44 and 47 years) were discovered to have peripheral cytopenias, which lead to bone marrow biopsy and flow cytometry studies.

Results: One patient had normocytic anemia and severe neutropenia (Hgb 9.8 g/dL; absolute neutrophil count, 388/μL). The second patient had normocytic anemia, severe neutropenia, and moderate thrombocytopenia (Hgb 6.8 g/dL; absolute neutrophil count, 100/μL; platelet count, 107,000). Core bone biopsies were mildly hypocellular in case 1 and hypercellular in case 2, but they were otherwise unremarkable. In both cases, aspirate smears were significant for immature granulocyte vacuolization and immature erythroid vacuolization as well as ringed sideroblasts (up to 5%), and one case had hemosiderin-laden plasma cells. Changes also included myeloid left-shift, mild megaloblastic change, and mild dyserythropoiesis. In both cases, flow cytometry revealed increased phenotypically normal hematogones (2.4% for case 1; 5.3% to 11% for case 2). In both cases, ceruloplasmin and copper levels were decreased, and zinc levels were increased. Copper supplementation lead to normalization of hematologic findings.

Conclusions: In patients with peripheral blood cytopenias, increased bone marrow hematogones accompanied by immature granulocyte and erythroid vacuolization should prompt clinical evaluation for copper deficiency.

Reviewer's Comments: In both of these cases, copper deficiency appeared to be secondary to excessive dietary zinc supplementation. Bone marrow cellular vacuolization is not specific for copper deficiency and has been reported in association with ethanol toxicity, drug effects, hematologic neoplasms, and chemotherapy.

Additional Keywords: None

Print Tag: Refer to original journal article
High Percentage of MCCs Express Merkel Cell Polyomavirus

Merkel Cell Polyomavirus Expression in Merkel Cell Carcinomas and Its Absence in Combined Tumors and Pulmonary Neuroendocrine Carcinomas.
Busam KJ, Jungbluth AA, et al:

Merkel cell carcinomas (MCC) associated with polyomavirus may have specific immunohistochemical features. IHC with antibodies to MCV-Associated TA and CK20 is very helpful for distinguishing MCC.

**Background:** Merkel cell carcinoma (MCC) is an uncommon high-grade neuroendocrine carcinoma of the skin. Neuroendocrine differentiation is generally easy to document with immunohistochemical (IHC) stains to chromogranin and synaptophysin. The tumors also frequently show immunoreactivity with antibodies to CK20. This is especially useful as it helps to distinguish these tumors from metastatic high-grade neuroendocrine carcinomas, especially small cell carcinomas, which are typically not immunoreactive with antibodies to CK20. Sun exposure and immunosuppression are considered risk factors for the development of MCC. Recently, it has been shown that a novel polyomavirus (Merkel cell polyomavirus [MCV]) is clonally integrated into a high percentage of MCCs. This integration leads to the expression of a specific tumor antigen (MCV-Associated TA).

**Objective:** To review MCV and MCV-Associated TA in a large number of MCCs and other neuroendocrine carcinomas.

**Methods:** Frozen MCC tissue was used for PCR studies for the MCV. Paraffin-embedded tissue was used for IHC, which was performed with antibodies to CK20 and MCV-Associated TA. A tissue array with MCCs, other neuroendocrine skin carcinomas, and pulmonary small cell carcinomas was also used for IHC.

**Results:** MCCs from 17 patients were available for PCR. MCV was detected in 88% of cases. By IHC, 67% of the overall cases expressed MCV-Associated TA, which represented 77% of the PCR-positive cases. Immunoreactivity with antibodies to CK20 was seen in 93% of cases. With the tissue array, 75% of MCCs were immunoreactive with antibodies to MCV-Associated TA, and 89% expressed CK20. Of the combined squamous cell carcinoma/neuroendocrine carcinomas and pulmonary small cell carcinomas, none expressed MCV-Associated TA, whereas 100% of the combined tumors and no pulmonary small cell carcinomas were immunoreactive with antibodies to CK20.

**Conclusions:** MCCs frequently have a novel integrated polyomavirus. IHC with antibodies to MCV-Associated TA and CK20 is very helpful for distinguishing MCC from pulmonary small cell carcinoma. Tumors found to be negative by PCR for MCV do not express the MCV-Associated TA by IHC.

**Reviewer's Comments:** This study confirms the fact that a high percentage of MCCs are associated with the MCV. Furthermore, it shows that IHC can identify most of these tumors.

Additional Keywords: None

Print Tag: Refer to original journal article
CD Treatment Not Warranted for Isolated Asymptomatic Ileitis

Isolated Asymptomatic Ileitis Does Not Progress to Overt Crohn Disease on Long-Term Follow-Up Despite Features of Chronicity in Ileal Biopsies.


Symptoms are the best predictor that isolated ileitis will progress to Crohn disease. At this time, patients with isolated asymptomatic ileitis do not appear to need treatment for Crohn disease.

Background: Ileitis is a common finding on biopsy and is sometimes, but not always, associated with Crohn disease (CD). Up to 5% of ileal biopsies show findings of ileitis, even when patients with suspected CD are excluded. It is believed that some of these patients will eventually progress to CD.

Objective: To review the endoscopic and morphologic findings seen in patients with isolated, asymptomatic ileitis and to determine the percentage of patients who progress to CD. Furthermore, specific features were sought that would help predict which patients would progress to CD.

Methods: A single institution's surgical pathology database was reviewed for cases in which endoscopic and histologic features of ileitis were noted. Patients with prior history of CD or other gastrointestinal findings consistent with CD were excluded. Only patients with ≥2 years of follow-up were included. Architectural features as well as stromal and inflammatory changes were noted, including villous blunting and crypt disarray, granulomas, extent of acute inflammatory infiltrate, character of chronic inflammatory infiltrate, fibromuscular proliferation, and pyloric gland metaplasia. Results were compared to the follow-up development of CD.

Results: 29 patients met the study criteria, including 14 who were asymptomatic and 15 who had symptoms (abdominal pain, diarrhea, bleeding, etc). The study population included 22 men and 7 women. Thirteen of 14 asymptomatic patients were on medication at the time of their biopsies, whereas 9 of the 15 symptomatic patients were receiving some medication on a regular basis. Endoscopic findings were similar in the symptomatic and asymptomatic patients. Pathologic findings revealed that most symptomatic and asymptomatic patients demonstrated features of chronic injury. Most asymptomatic patients noted to have pyloric gland metaplasia did not progress to CD during follow-up, while 10 of 15 symptomatic patients progressed to CD. Symptomatic patients with pyloric gland metaplasia were very likely to progress to CD (5 of 6 patients). No specific histologic features were helpful for distinguishing who would progress to CD, except for granulomas, which were seen only in 1 case.

Conclusions: Isolated ileitis in asymptomatic patients does not progress to CD, even when the morphologic features of chronicity are present.

Reviewer's Comments: This is a good paper that methodically reviews the histologic changes seen with ileitis. It will be interesting to see if longer follow-up shows more patients with pyloric metaplasia progressing to CD.

Additional Keywords: None

Print Tag: Refer to original journal article
Consider Using Excision Specimens to Repeat HER-2/neu Tests

Comparison of Fluorescent In Situ Hybridization HER-2/neu Results on Core Needle Biopsy and Excisional Biopsy in Primary Breast Cancer.

Apple SK, Lowe AC, et al; Mod Pathol 2009; 22 (September): 1151-1159

Especially in breast cancer cases with tumor variability, repeating HER-2/neu studies on excisional samples may be prudent in cases that have undergone neoadjuvant therapy and in cases with borderline results from core biopsy.

Background: HER-2/neu receptor status in breast cancer is an important pathologic assessment with therapeutic implications. Trastuzumab (Herceptin®) is a target-driven oncological drug that is administered to patients demonstrated to be positive for HER-2/neu overexpression by immunohistochemistry (IHC) or by amplification via fluorescent in situ hybridization (FISH). Receptor studies may be performed on the core needle biopsy (CNB) if there is sufficient tissue. However, there is no recommendation for repeating the test on subsequent excisional biopsies. Objective: To determine the correlation between IHC and FISH results from CNB and excisional biopsy and to re-evaluate discrepant results.

Methods: 125 individual patients with invasive carcinoma in both CNB and excisional specimens (lumpectomy or mastectomy) were identified, 5 of whom received intervening neoadjuvant chemotherapy. All cases were subjected to routine IHC for estrogen receptor, progesterone receptor, Ki-67, HER-2/neu (HercepTestTM kit) and FISH for HER-2/neu (Vysis PathVysion®) and all were scored independently.

Results: Concordance of the CNB and subsequent excisional biopsy by IHC results was 98% and by FISH results was 92%. Concordance of the IHC results compared to FISH results was 95% across all specimens. Complete IHC results and FISH results from both the CNB and subsequent excisional biopsy had a concordance rate of 87%. All of the specimens from patients who received interval neoadjuvant chemotherapy had complete concordance of HER-2/neu results. Review of discrepant results across CNB to the subsequent excision determined 2 cases with a higher-grade lesion detected on excision specimen than was observed in the CNB. Repeated review of available slides from cases with discrepant results across IHC methods to FISH methods revealed 12 of 30 cases with an IHC result that would have required a reflex FISH test for definitive evaluation by the 2007 American Society of Clinical Oncology/College of American Pathologists criteria.

Conclusions: Neither IHC nor FISH methods for detecting HER-2/neu overexpression/amplification have perfect concordance across CNB and subsequent excisional specimens. Cases which may warrant consideration for repeat studies would include those with a small tumor sample in the CNB or intratumoral heterogeneity noted in subsequent excision, borderline or equivocal results in the CNB, or specimens from patients who have undergone neoadjuvant chemotherapy.

Reviewer’s Comments: Some others have suggested repeating the HER-2/neu studies on biopsies of metastatic breast cancer for potential changes in receptor status that could impact treatment options.

Additional Keywords: None

Print Tag: Refer to original journal article
Nuclear DRP1 Expression Confers Poor Prognosis in Lung Cancer

Nuclear Expression of Dynamin-Related Protein 1 in Lung Adenocarcinomas.
Chiang YY, Chen SL, et al.:
Mod Pathol 2009; 22 (September): 1139-1150

Nuclear DRP1 expression is highly expressed in adenocarcinomas of the lung and confers a poor prognosis, which may be related to chemoresistance.

**Background:** The dynamin superfamily includes dynamin-related protein 1 (DRP1) and is involved in intercellular processes such as budding and scission of vesicles and organelles. Along with mitochondria, these proteins are responsible for programmed cell death and have implications in cancer progression. Many chemotherapeutic drugs target such processes to accelerate steps in cellular death, but mitochondrial fragmentation may be a component of the side effects.

**Objective:** To study DRP1 expression in adenocarcinomas of the lung with correlation to clinicopathologic features (including outcome) and to investigate effects of hypoxia on expression and drug resistance in vitro.

**Methods:** 227 patients diagnosed with adenocarcinoma of the lung were collected, all of whom had undergone surgical resection and postoperative cisplatin-based chemotherapy. Lung cancer cell lines and a cervical cancer cell line were also used for in vitro studies of induced hypoxia. Immunohistochemical stains for DRP1 were produced and performed on the tissue samples, with validation by immunoblotting.

**Results:** DRP1 overexpression was demonstrated in 89.0% of the adenocarcinomas, mostly noted in the nuclei of tumor cells (91.1%). Metastatic disease in lymph nodes was also predominantly positive for DRP1 (91.2%). The overexpression of DRP1 was positively correlated to tumor stage and a history of cigarette smoking. Of the patients with nuclear DRP1 overexpression, the tumor recurred in 56.9% during the follow-up, which was a rate 3.41 times higher than that seen in patients with cytoplasmic DRP1 overexpression. Induced hypoxia on cell lines resulted in increased nuclear DRP1 levels and increased cisplatin resistance, while silencing of protein hHR23A decreased nuclear DRP1 levels and decreased cisplatin resistance.

**Conclusions:** Nuclear DRP1 is overexpressed in most adenocarcinomas of the lung, which corresponds to higher stage, smoking history, and increased risk of tumor recurrence. Hypoxic conditions may increase resistance to common chemotherapeutic agents, while translocation of this protein to the nucleus may be interrupted with hHR23A.

**Reviewer's Comments:** By examining cellular proteins and intercellular actions in response to exposure to chemotherapy, we may improve our understanding of therapeutic failures.

Additional Keywords: None

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