CGH Less Sensitive Than FISH for CLL Genomic Profiling

Customized Oligonucleotide Array-Based Comparative Genomic Hybridization as a Clinical Assay for Genomic Profiling of Chronic Lymphocytic Leukemia.
Sargent R, Jones D, et al:
J Mol Diagn; 2009; 11 (January): 25-34

Array based comparative genomic hybridization is slightly less sensitive than FISH for characterizing known genetic alterations in chronic lymphocytic leukemia, but theoretically, it may detect random genetic abnormalities.

Background: Among chromosomal deletions and gains that are prognostically significant in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), unfavorable 17p deletions are the strongest predictor of outcome. Genetic abnormalities with lesser prognostic significance include del 11q22.3, trisomy 12, del 13q14, and an additional locus as chromosome 13q34, all of which are routinely assayed by FISH. Comparative genomic hybridization (CGH) of labeled tumor DNA to microarrays, as opposed to metaphase spreads, provides increased resolution, sensitivity, and lab throughput.

Objective: To evaluate high-density CGH arrays for characterizing genetic alterations in CLL/SLL.

Methods: 100 samples of CLL, including 62 for a pilot study (56 bone marrow; 6 peripheral blood) and 38 subsequent sequential cases (26 bone marrow; 12 peripheral blood) were analyzed by FISH at 11q22.3, 12p11.1-q11, 13q14.3, 13q34, and 17p13.1. Isolated patient and control female DNA were digested and labeled with Cy5-dUTP or Cy3-dUTP, respectively, prior to hybridization to a customized 44,000-probe oligonucleotide array. The array represented human genomic DNA at a resolution of 50 to 75 kb, except for loci known to be abnormal in CLL, for which high density probe tiling produces a resolution of 5 to 11 kb.

Results: In the pilot series, 56 cases had array CGH and FISH data for comparison. Among these, there was 93% concordance for del 11q22.3, 92% for trisomy 12, 78% concordance for del 13q14.3, 50% concordance for del LAMP1/13q34, and 72% concordance for del TP53/17p13.1. In the sequential series, among 36 cases with array CGH and FISH data for comparison, there was 89% concordance between array CGH and FISH. There were no false positives by array CGH. Overall, among the 100 samples, array CGH and FISH were 89% concordant. Most discordance (84%) occurred in specimens with <25% CLL cells, which appears to be a limit of array CGH sensitivity.

Conclusions: Array based CGH appears to be slightly less sensitive than FISH for characterizing known genetic alterations in CLL but has the theoretical advantage of detecting random genetic abnormalities.

Reviewer's Comments: Array based CGH detects only unbalanced genetic changes (chromosomal gain or loss). Balanced genetic changes that characterize many leukemias and lymphomas, such as reciprocal translocations and inversions, are not detected. Therefore, one can predict a role for array based CGH in evaluation of diseases such as myelodysplastic syndromes, but it is unlikely to replace FISH testing for many recurrent leukemias and lymphoma-associated changes. (Reviewer-Guy E. Nichols, MD).

print tag: () Refer to original journal article.
HPV Positivity Improves Prognosis for Sinonasal SCCs

Human Papillomaviruses Are Identified in a Subgroup of Sinonasal Squamous Cell Carcinomas With Favorable Outcome.

Alos L, Moyano S, et al:
Cancer; 2009; 115 (June 15): 2701-2709

The subset of squamous cell carcinomas of the sinonasal tract that is associated with human papillomavirus (HPV) infection has a significantly better prognosis that do HPV-negative tumors.

Background: Human papillomavirus (HPV) has been implicated as an important agent in the development of squamous cell carcinoma (SCC) of the uterine cervix. Significant epidemiologic and molecular data support the role of HPV in the development of some head and neck SCCs, particularly in the oropharyngeal region. Importantly, HPV-positive SCCs of the oropharynx have a better prognosis than HPV-negative tumors from the same area. Limited evidence for HPV-related SCC at other head and neck sites is available.

Objective: To determine the clinical implications of HPV-positive sinonasal SCCs.

Methods: 60 patients with histologically confirmed SCC of the sinonasal tract were included. Clinical and pathologic data were extracted from the medical records and pathology reports, including age, tobacco use, tumor site, tumor stage, and survival data. DNA was extracted from a representative block of the tumor in each case. Broad-spectrum HPV DNA amplification was performed and then tested with an HPV line-probe assay to determine the specific genotype, if present. Immunohistochemistry for p16INK4a was performed on each case. Only cases with diffuse nuclear p16 staining were considered positive.

Results: 12 tumors (20%) were HPV-positive by PCR, of which 11 (92%) were HPV type 16 and 1 harbored HPV type 35. P16 had a 100% sensitivity and specificity for the detection of HPV-positive tumors. There was no statistical difference in age, gender, or tumor stage between HPV-positive and HPV-negative SCCs. Tobacco use was significantly higher in patients with HPV-negative SCCs. HPV-positive SCCs more commonly arose in the nasal cavity (92%) than the paranasal sinuses alone (8%). The 5-year progression-free survival rate for HPV-positive and HPV-negative patients was 62% and 20%, respectively. The 5-year overall survival rate for HPV-positive and HPV-negative patients was 80% and 31%, respectively.

Conclusion: A subset of sinonasal SCCs are associated with HPV infection. These patients have a significantly better prognosis than do those with non-HPV-related tumors at the same site.

Reviewer’s Comments: The results of this well-designed study are similar to those of prior oropharyngeal SCC studies of HP infection. The subset of patients with HPV-positive tumors of the sinonasal tract showed a significantly improved prognosis. P16 is a good marker for HPV infection in these tumors when a criterion of diffuse, strong staining is used. (Reviewer-Deborah J. Chute, MD).

print tag: () Refer to original journal article.
New Plasma-Based Biomarker Assesses Colorectal Cancer Risk

Circulating Methylated SEPT9 DNA in Plasma Is a Biomarker for Colorectal Cancer.

deVos T, Tetzner R, et al:
Clin Chem; 2009; 55 (July): 1337-1346

**SEPT9** is a recently described DNA methylation-based biomarker whose measurement in plasma samples may be a useful screening test for colon cancer.

**Background:** A goal of the American Cancer Society is to appropriately screen 75% of eligible persons for colorectal cancer by the year 2015. Current screening guidelines recommend using a variety of screening tests, including optical colonoscopy, sigmoidoscopy, fecal occult blood testing, and fecal DNA testing. The development of a simple, reliable, and noninvasive blood-based test for the assessment of colon cancer risk would provide an important testing methodology, especially for those unable to undergo or afford invasive procedures. Aberrantly methylated DNA has been detected in a number of solid tumors, including colon cancer, and detection of this aberrantly methylated DNA in plasma using PCR assays has been reported. One of these novel DNA methylation-based biomarkers is called septin 9 (SEPT9).

**Objective:** To report the development and validation of a new assay for the detection of SEPT9 methylated DNA in plasma samples from patients with colon cancer.

**Methods:** The study included 97 patients with colon cancer and 172 healthy controls. The cancer patients had varying stages of colon cancer, but none had a history of HIV, herpesvirus B or C infection, or other cancer (except basal cell carcinoma). Blood samples were collected, and plasma was prepared from each sample within 4 hours of collection. The SEPT9 methylation assay included a number of sequential steps: DNA magnetic particle-based extraction, DNA bisulfite conversion using a thermocycler in an overnight reaction, bisulfite DNA purification, product amplification using the LightCycler, and curve detection with manual review and result interpretation. Positive and negative controls were included with each extraction and subsequent analysis.

**Results:** Following bisulfite conversion of plasma DNA, the SEPT9 assay detected a mean of 1.9 g/L of plasma DNA, representing an approximate recovery of 50% of sample genomic DNA. In the testing study, the assay detected 68% of cancers with a specificity of 89%.

**Conclusions:** The measurement of circulating methylated plasma DNA (SEPT9 DNA), as described using this novel method, is useful as a biomarker for detecting minimally invasive colon cancer. Unlike previous testing methodologies, the current assay is well-suited for application in most molecular diagnostic laboratories.

**Reviewer's Comments:** Although still in its infancy, the field of plasma DNA detection in patients with solid malignancies is rapidly developing. The current study presents a refined assay that appears to overcome many of the challenges encountered with previously reported plasma DNA biomarker assays. As the authors report, their refined assay has apparently eliminated PCR inhibition, simplified specimen handling steps, increased automation potential, and reduced testing costs. (Reviewer-T. David Bourne, MD).

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MAP-2 One of First Specific Markers for Ganglion Cells


Burtelow MA, Longacre TA;
Am J Surg Pathol; 2009; 33 (July): 1025-1030

Microtubule associated protein-2 (MAP-2) immunostaining may be a helpful adjunct for the identification of ganglion cells in rectal biopsies.

Background: Hirschsprung's disease (HD) is characterized by segmental absence of colonic ganglion cells secondary to a lack of cellular migration. For diagnosis, suction biopsy is considered the gold standard, and the identification of ganglion cells within the submucosa effectively excludes the disease. Sometimes, the biopsies do not contain enough submucosal tissue - up to 6% are considered inadequate. Because of the implications of a diagnosis of HD, protocols for the assessment of rectal suction biopsies suggest the evaluation of numerous profiles (up to 100) and sometimes suggest the use of adjunct testing, including acetylcholinesterase staining for the identification of thickened cholinergic nerves present in aganglionic colon specimens.

Objective: To evaluate the use of immunohistochemistry for microtubule-associated protein-2 (MAP-2; a cytoplasmic microtubule stabilizing protein) for the identification of ganglion cells in rectal suction biopsies.

Methods: A single institution's surgical pathology database was searched and identified 169 biopsies of possible HD, of which 78 had shown ganglion cells and 91 had not shown ganglion cells. An additional 78 biopsies were considered suboptimal. The biopsies had been exhausted and leveled onto 20 slides. A representative slide from each case was selected, the coverslip removed, and the slide was submitted for MAP-2, S100, and CD117 immunostaining.

Results: MAP-2 staining was first demonstrated to be specific for ganglion cells in the pediatric bowel through staining of normal and aganglionic bowels. Only ganglion cells showed any staining. All biopsy samples that had been designated to have ganglion cells showed ganglion cells by MAP-2 staining. Of the 91 biopsies that were noted to not have ganglion cells by HE, 3 showed rare, solitary staining cells in patients who were later found not to have HD, suggesting that the staining identified 3 cases with ganglion cells that had been missed by HE. Of the 78 insufficient or equivocal cases, the 28 that contained no submucosa showed no staining with MAP-2. Of the remaining 50 cases, MAP-2 identified ganglion cells in 8 cases. All patients with follow-up and with biopsies found to have ganglion cells with MAP-2 immunostaining were found not to have HD.

Conclusions: MAP-2 appears to be a sensitive and specific marker for the identification of ganglion cells in rectal biopsies and may be especially helpful with specimens that are equivocal.

Reviewer's Comments: MAP-2 appears to be one of the first truly specific markers for the identification of ganglion cells. Further studies confirming the findings here may help redefine protocols for the assessment of rectal suction biopsies. (Reviewer-Edward B. Stelow, MD).

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T-Cells May Indicate Improved Survival in Endometrial Cancer

**Presence of Tumor-Infiltrating Lymphocytes Is an Independent Prognostic Factor in Type I and II Endometrial Cancer.**

*de Jong RA, Leffers N, et al: Gynecol Oncol; 2009; 114 (July): 105-110*

T-cell infiltration of endometrial cancer is associated with prognostic significance that may indicate an important role of immune modulation.

**Background:** Regulatory T-cells (Treg) play a role in immune response and are known to be increased in patients with various malignancies, corresponding to a worse prognosis. Tumor-infiltrating lymphocytes, especially cytotoxic T cells (CTL), have been noted to associate with a better prognosis in several cancers. In addition, a high CTL/Treg ratio is correlated with an improved prognosis in ovarian and cervical cancers. In colorectal cancers, memory T-cells are associated with lower stage of presentation and better prognosis. The prognostic value of these T-cell subsets has not been explored in endometrial cancers.

**Objective:** To evaluate the presence and prognostic value of T-cell subsets in endometrial cancer.

**Methods:** Patients with endometrial cancer were identified, and tissue blocks were obtained to produce a microarray of tumor samples. Immunohistochemistry was performed for CD8, FoxP3, and CD45RO. Infiltrating T-lymphocytes were then quantified for each tumor and correlated to clinicopathologic features, including survival.

**Results:** 368 patients treated from 1984 to 2004 were included in the array. They had an average age of 65 years (range, 32-89 years) and an average follow-up of 4.4 years (range, 0-21.5 years). Most cases were well-differentiated to moderately differentiated and early stage disease. Of the 368 cases, 59 (16%) died of disease. CD8+, FoxP3+, and CD45RO+ T-cells were present in 96.8%, 79.4%, and 60.7% of tumors, respectively. CD45RO+ results were binary and their presence correlated with favorable clinicopathologic features. Higher numbers of CD8+ cells and a high ratio of CD8+/FoxP3+ cells were also associated with favorable clinicopathologic features and longer disease-free survival. The presence of CD45RO+ cells and higher numbers of CD8+ cells also corresponded to a better overall survival. In a multivariate analysis, the presence of CD45RO+ cells and higher numbers of CD8+ T-cells were independent prognostic factors for the entire cohort, while a high ratio of CD8+/FoxP3+ cells was an independent prognostic factor for type I endometrial cancer only.

**Conclusions:** The presence of T-lymphocytes in endometrial cancers is a prognostic factor that may relate to a role of the immune system in this cancer, like many others.

**Reviewer’s Comments:** The authors demonstrate how the immune system likely plays an important role in endometrial cancer (like colonic, ovarian, and others). Future studies should take advantage of this role and explore new therapeutic strategies in these cancers. (Reviewer-Mary T. Galgano, MD).

**print tag:** (Refer to original journal article.)
Unique Fusion Gene Prevalent in Peripheral Zone Prostatic Cancer

**Prostate Cancer of Transition Zone Origin Lacks TMPRSS2-ERG Gene Fusion.**

Guo CC, Zuo G, et al: 
*Mod Pathol*; 2009; 22 (July): 866-871

The fusion gene **TMPRSS2-ERG** is found in peripheral rather than transitional zone prostate cancers.

**Background:** Recent research has identified a unique chromosomal rearrangement in a percentage of prostatic adenocarcinomas: the fusion of the 5'-transmembrane protein serine proteinase-2 (TMPRSS2) with the oncogene EST-related gene (ERG). Although the clinical significance of this fusion product remains unclear, some evidence suggests that its presence in prostate cancer may be associated with a more aggressive phenotype.

**Objective:** To investigate the association between TMPRSS2-ERG gene fusion status and the zonal origin of prostatic adenocarcinoma.

**Methods:** The authors retrospectively searched the M.D. Anderson Cancer Center's pathology archives between 2001 and 2008 for radical prostatectomy specimens using 2 inclusion criteria: the specimen had to contain at least 2 tumor foci, and both the peripheral and transition zones had to contain tumor. Thirty patients met both criteria. The largest area from each involved zone was selected for study in cases with >1 independent tumor focus per zone. Each selected tumor focus was assigned a Gleason score, the volume of tumor was recorded, and the presence or absence of the TMPRSS2-ERG gene fusion was assessed using break-apart FISH.

**Results:** The mean patient age was 59 years (range, 46 to 71 years). The mean Gleason score was 7.0 (range, 6.0 to 8.7). The tumor was confined to the prostate in 23 cases and showed evidence of extraprostatic extension in 7. Transition zone adenocarcinomas tended to show large irregular glands lined by relatively tall columnar cells with basally oriented nuclei. In contrast, peripheral zone adenocarcinomas were more likely to be composed of small round glands lined by cuboidal epithelial cells. Foci of tumor from the transition zone had a mean Gleason score of 6.8 and a mean tumor volume 1.2 cm\(^3\). Tumor from the peripheral zone had a mean Gleason score of 6.7 and a mean tumor volume of 4.0 cm\(^3\). All transition zone tumors showed normal FISH signal patterns for ERG, with no evidence of gene rearrangement. Among peripheral zone cancers, however, 13 cases (43%) showed ERG rearrangements.

**Conclusions:** The TMPRSS2-ERG gene fusion is highly prevalent in prostatic adenocarcinomas that arise in the peripheral zone rather than the transition zone.

**Reviewer's Comments:** As the authors point out, it is accepted that many so-called transition zone adenocarcinomas generally have a more favorable prognosis compared to their peripheral zone counterparts. This is true despite the somewhat paradoxical observation that transition zone tumors tend to be larger. The finding that this unique gene fusion product may be restricted to peripheral zone adenocarcinomas may partly explain the underlying biological difference between tumors arising in this zone versus the transition zone. (Reviewer-Stacey E. Mills, MD).

**print tag:** () Refer to original journal article.
Management Controversial When Find Isolated Tumor Cells in SLN

Non-Sentinel Lymph Node Metastases Associated With Isolated Breast Cancer Cells in the Sentinel Node.

van Deurzen CH, de Boer M, et al:

*J Natl Cancer Inst*; 2008; 100 (November 19): 1574-1580

Isolated breast cancer tumor cells in SLNs predict non-SLN metastases in 10% to 16% of patients, most of whom will have non-SLN macrometastases and are candidates for adjuvant systemic therapy.

**Background:** Sentinel lymph node (SLN) status is a widely accepted, less morbid but acceptably accurate surrogate predictor of nodal status compared to axillary lymph node dissection and is the favored method of breast cancer staging. When SLNs are positive for histologically detectable micrometastasis or macrometastasis, patients undergo axillary lymph node dissection, either in the same surgical procedure when detected by frozen section or in a second surgical procedure. However, the clinical significance and management implications of isolated breast cancer tumor cells (tumor cell clusters measuring <0.2 mm regardless of detection method) remain controversial.

**Objective:** To review the value of isolated breast cancer tumor cells in SLNs as predictors of the status of non-SLNs and to recommend management of patients with isolated breast cancer tumor cells in SLN biopsies.

**Methods:** 411 published articles were identified, and 29 were selected for which all subjects had invasive breast cancer (microinvasive excluded), SLN biopsy followed by axillary node dissection, and SLNs with isolated tumor cells defined by the sixth edition of the AJCC Cancer Staging Manual. These articles comprised 836 patients.

**Results:** Among 836 patients with SLNs containing isolated tumor cells, 108 had non-SLN metastases, resulting in an overall risk of 12.3% (95% CI = 9.5% to 15.7%). A major portion of these non-SLN metastases were macrometastases (pooled risk, 63.5%) which would require adjuvant therapy. Authors of the two largest studies recommended axillary lymph node dissection for breast cancer patients with SLNs containing isolated tumor cells, with only 1 author group suggesting that pT1a/pT1b tumors or pT1 tubular, colloidal, and medullary tumors might forgo axillary dissection.

**Conclusions:** Isolated breast cancer tumor cells in SLNs predict non-SLN metastases in 10% to 16% of patients, most of whom will have non-SLN macrometastases.

**Reviewer's Comments:** The authors suggest that patients with SLN isolated tumor cells be considered for axillary lymph node dissection, depending on other clinicopathologic features. It is possible that this meta-analysis is biased toward patients with isolated tumor cells and worrisome clinical features that were not captured here, which might over-exaggerate the clinical risk of isolated tumor cells. (Reviewer-Guy E. Nichols, MD).

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CISH Highly Concordant With FISH for HER2 Amplification

The Correlation Between Dual-Color Chromogenic In Situ Hybridization and Fluorescence In Situ Hybridization in Assessing HER2 Gene Amplification in Breast Cancer.

Pedersen M, Rasmussen BB: Diagn Mol Pathol; 2009; 18 (June): 96-102

Dual-color chromogenic in situ hybridization demonstrated a high concordance with FISH in detecting HER2 amplification in breast cancer.

Background: Amplification of the human epidermal growth factor receptor 2 (HER2) gene is a prognostic marker and therapeutic target for breast cancer. FISH is the current gold standard for assessing HER2 status, but it requires specialized equipment and training. In addition, the signals fade over time, and identifying tumor cells can be difficult using this method. Chromogenic in situ hybridization (CISH) is a new method which uses color detection rather than fluorescence, allowing assessment of HER2 amplification with light microscopy.

Objective: To report the authors’ experience with dual-color CISH for simultaneous detection of the HER2 gene and the centromere of chromosome 17 (CEN-17).

Methods: 80 breast cancers with known HER2 status by immunohistochemistry, representing all categories of HER2 expression, were included. Tissue microarrays were constructed for FISH and CISH analysis. FISH was performed with the FDA-approved test kit HER2 FISH pharmDx, using the HER2/CEN-17 Probemix. CISH was performed using the HER2 FISH pharmDx kit and the HER2/CEN-17 Probemix, but to visualize the FISH probes on light microscopy, anti-biotin/AP and anti-FITC/HRP antibodies were used. A red chromogen was used to detect the AP signal (HER2), and a blue chromogen was used to detect the HRP signal (CEN-17). FISH and CISH were scored in at least 20 cells per tumor, and the number of red and blue signals for each cell was recorded. A HER2/CEN-17 ratio was performed, and HER2 amplification was defined as a ratio >=2. If ratios were between 1.8 and 2.2, then an additional 20 tumor cells were scored, and the ratio was recalculated.

Results: Paired results were available in 72 cases. FISH detected HER2 amplification in 25 (35%) cases, and CISH detected HER2 amplification in 24 (33%) cases, with an agreement of 98.6%. HER2/CEN-17 ratios >2 were on average higher when using the CISH method. The increase in HER2/CEN-17 ratios by CISH in amplified cases was possibly due to hidden red or blue signals, a problem not encountered with FISH. No nonspecific red or blue staining was seen in the CISH slides. CISH signals were distinguishable and could be counted without difficulty.

Conclusion: Dual-color CISH demonstrated a high concordance with FISH in detecting HER2 amplification. Dual-color CISH is a promising alternative to the FISH method, and it will also detect chromosome 17 polysomy on the same slide.

Reviewer's Comments: Single-color CISH typically will be performed on 2 separate slides for HER2 gene and CEN-17 detection, and has been shown to be highly concordant with FISH analysis. (Reviewer-Deborah J. Chute, MD).

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New Neutrophil Threshold Identifies Septic Joint Loosening

Twenty-Three Neutrophil Granulocytes in 10 High-Power Fields Is the Best Histopathological Threshold to Differentiate Between Aseptic and Septic Endoprosthesis Loosening.

Morawietz L, Tiddens O, et al:
Histopathology; 2009; 54 (June): 847-853

Based on ROC-curve analysis, 23 neutrophils in 10 HPFs is the proposed threshold for rendering a diagnosis of septic endoprosthesis loosening.

**Background:** After total hip or knee replacement surgery, approximately 10% of joints will develop septic or aseptic endoprosthetic loosening. Distinguishing between these causes has important treatment implications. Criteria-based pathologic evaluation of periprosthetic membrane tissue remains controversial. Disagreement persists in the literature regarding the number of neutrophils per high-power fields (HPF) required to diagnose septic loosening.

**Objective:** To determine a threshold number of neutrophils required to distinguish between septic and aseptic endoprosthesis loosening.

**Methods:** The authors studied periprosthetic membrane tissue excised from 147 patients who underwent endoprosthesis revision surgery. Of the joint loosenings, approximately 66% were clinically diagnosed as aseptic, 3% as suspicious, and 31% as septic. Clinical criteria for a diagnosis of sepsis included one or more of the following findings: presentation with signs and symptoms of infection, positive radiographic findings, elevated C-reactive protein levels (>0.5 mg/dL), elevated white blood cell count (>12/nl), early prosthetic failure (within first 5 years), and positive joint aspiration. Six samples from each joint were sent for microbiological culture using a wide variety of inoculation media. Tissue samples were also routinely processed for HE staining and PAS special staining. Immunohistochemical staining for CD15 was also performed for each case. Examination using light microscopy began with low-power examination to identify the area richest in neutrophils. These areas were then examined using 400X magnification for counting neutrophils present within tissue. Ten HPFs were assessed.

**Results:** ROC-curve analysis showed an optimal threshold of 23 neutrophils per 10 HPF, with a sensitivity of 73% and a specificity of 95% when compared with the microbiological culture results. When compared with the clinical diagnosis, this threshold value had a sensitivity of 77% and a specificity of 97%. PAS led to under-recognition of neutrophils compared with CD15 immunostaining in difficult-to-count cases.

**Conclusions:** The recommended threshold value for the histological diagnosis of septic joint loosening is >=23 neutrophils per 10 HPFs. CD15 immunostaining, rather than PAS staining, is recommended as an aid to counting in difficult-to-examine cases.

**Reviewer’s Comments:** The search for neutrophils in periprosthetic tissue is often tedious, especially when large tissue samples are sent for intraoperative consultation. The recommendation in this article should be considered, but any change in diagnostic practice should first be discussed with the orthopedic surgery service at your institution to avoid confusion or miscommunication. (Reviewer-T. David Bourne, MD).

**print tag:** () Refer to original journal article.
Embolic therapy of tumors with radioactive beads can lead to iatrogenic damage of other organs.

**Background:** Novel treatments for hepatic malignancy now include selective internal radiation therapy (SIRT) using 90Yttrium-labeled microspheres. With this treatment, radiolabeled microspheres are deposited in the hepatic artery and embolize to the intrahepatic tumor. A number of previously published studies discuss extrahepatic tissue injury that usually develops after inadvertent extrahepatic embolization of the radioactive materials.

**Objective:** To describe 4 cases of extrahepatic injury secondary to SIRT, and to present a literature review regarding this phenomenon.

**Methods:** Four cases of SIRT-induced extrahepatic injury were seen at a single institution. Pretreatment and posttreatment clinical data were reviewed. Pathologic materials were reviewed. The literature was reviewed for reported cases of SIRT-induced extrahepatic injury.

**Results:** 3 women and 1 man (age range, 59-75 years) had SIRT-induced extrahepatic injury. Three patients had metastatic neuroendocrine carcinomas, and 1 had a metastatic rectal carcinoma. Patients presented with extrahepatic complaints between 1 and 11 months after treatment for their hepatic tumors with 90Yttrium-labeled microspheres. Three patients presented with epigastric pain, and one of these patients also had hematemesis, melena, and anemia. All had gastric ulcers which showed 90Yttrium-labeled microspheres with epithelial erosion and atypia. Stromal fibrosis was also seen. Two cases had an associated granulomatous reaction to the microspheres. One patient presented with acute right upper quadrant pain. She was noted to have gallstones and underwent cholecystectomy. Although she was noted to grossly have gallstones at resection, histologically an acute and chronic cholecystitis was present with surface epithelial erosion. Numerous intravascular black 90Yttrium-labeled microspheres were associated with a foreign body giant cell reaction. In the literature review, the authors noted a variable rate of associated extrahepatic toxicity as assessed by symptomatology. Most symptomatology noted was either systemic or gastric in nature, with cases of cholecystitis and pancreatitis also noted. When specifically addressed, the rate of gastric ulcer ranged from 1% to 14%.

**Conclusions:** Selective internal radiation therapy is promising because it allows, in theory, high doses of radiation therapy to be specifically directed to malignant disease. It is not without extrahepatic complications, however, due largely to embolization of radioactive material to other sites.

**Reviewer’s Comments:** The authors nicely summarize the literature regarding extrahepatic injury with SIRT. Pathologists should be aware of this phenomenon, especially when reviewing gastric ulcer specimens. (Reviewer-Edward B. Stelow, MD).

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p16 Staining Helpful in Distinguishing EEA, Endocervical Tumors

Scoring of p16INK4a Immunohistochemistry Based on Independent Nuclear Staining Alone Can Sufficiently Distinguish Between Endocervical and Endometrial Adenocarcinomas in a Tissue Microarray Study.

Han CP, Kok LF, et al:
Mod Pathol; 2009; 22 (June): 797-806

Nuclear staining with or without cytoplasmic staining of p16 in a diffuse pattern is characteristic of endocervical adenocarcinoma and may contribute to a panel intended to differentiate this from endometrial adenocarcinoma.

Background: Endometrial and endocervical adenocarcinomas can overlap in clinical presentation and histopathologic features. However, depending on stage of disease and other factors, treatment protocols may differ. Immunohistochemical studies to differentiate between endometrial and endocervical primaries have narrowed the panel to estrogen receptors (ER), progesterone receptors (PR), vimentin, and carcinoembryonic antigen (CEA). But, many cases do not clearly fall into the category of positive ER, PR, and vimentin with negative CEA (for endometrial) or negative ER, PR, and vimentin with a positive CEA (for endocervical). Given the penchant for p16-positive staining in endocervical carcinomas but not endometrial carcinomas, it may be a valuable addition to the diagnostic panel.

Objective: To evaluate p16 staining patterns in endometrial and endocervical carcinomas using small tissue samples (akin to a preoperative biopsy) for utility in distinguishing site of origin.

Methods: 21 endometrial endometrioid adenocarcinomas (EEAs) and 14 endocervical adenocarcinomas, endocervical type, were collected from hysterectomy specimens demonstrating a clear site of origin. The tumors were incorporated into a tissue microarray which was subjected to immunohistochemical staining for p16 and scored by multiple semiquantitative methods for analysis, including Method Nucleus, Method Dominant Cytoplasm or Nucleus, and Method Mean of Cytoplasm and Nucleus.

Results: In endocervical adenocarcinomas, p16 nuclear staining was predominant in 7 of 14, nuclear plus cytoplasmic stainings were codominant in 5 of 14, and cytoplasmic staining was predominant in 2 of 14 tumors. In EEA, p16 staining was negative in 3 of the 21 cases, while nuclear staining was predominant in 7 of 21, nuclear plus cytoplasmic stainings were predominant in 6 of 21, and cytoplasmic staining was predominant in 5 of 21 tumors. All but Method Cytoplasm were statistically significant in distinguishing between endocervical adenocarcinomas and EEA, but Method Mean of Cytoplasm and Nucleus was the most accurate (80%).

Conclusions: Endocervical adenocarcinomas tend to express nuclear with or without cytoplasmic p16 in a more diffuse manner than endometrial adenocarcinomas, but evaluation of the cytoplasmic staining alone does not distinguish between the two.

Reviewer's Comments: This study was restricted to conventional EEAs and endocervical-type endocervical adenocarcinomas. It did not explore the area of histomorphologic overlap. However, the addition of p16 into a panel of immunohistochemical stains may contribute to the distinction of EEA by primary evaluation of nuclear staining. (Reviewer-Mary T. Galgano, MD).

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p16, p53 May Help Identify STUMPs With Increased Recurrence Risk

Ip PP, Cheung AN, Clement PB:

Uterine smooth muscle tumors of uncertain malignant potential typically behave in a benign fashion.

Background: Uterine smooth muscle tumors are usually easily designated as benign or malignant. Leiomyomas typically have low mitotic activity, little nuclear or cytologic atypia, and no tumor cell necrosis. However, leiomyosarcomas typically have >10 mitotic figures per 10 high-powered fields (HPFs), moderate to severe atypia, and tumor cell necrosis. Those tumors that are considered equivocal are termed "smooth muscle tumors of uncertain malignant potential (STUMPs)."

Objective: To review the clinicopathologic features of 16 STUMPs and to identify additional clinicopathologic features that may be helpful for predicting the behavior of these tumors.

Methods: 16 cases diagnosed as STUMP during a 15-year period were identified from multiple institutions and included atypical leiomyoma with limited experience (n=6), atypical leiomyoma with low risk of recurrence (n=2), smooth muscle tumor of low malignant potential (n=7), and mitotically active leiomyoma with limited experience (n=1). Clinical and follow-up data were gathered. All tumors had at least one section taken per centimeter of tumor. Cellularity, cytologic atypia, mitotic activity, and type and degree of necrosis were assessed. Tumors were immunostained with antibodies to p53, p16, ER, PR, and ki67.

Results: The patients ranged in age from 25 to 64 years. Patients typically presented with bleeding and mass symptoms. All but one patient underwent hysterectomy. All tumors were confined to the uterus with no extrauterine disease at resection. Tumors ranged in size from 0.7 to 18 cm. Most were intramural and circumscribed. Necrosis was grossly evident in most cases. Microscopically, all tumors appeared circumscribed, and no angiolympathic invasion was seen. Thirteen cases were hypercellular, and 15 cases had mitotic activity with a mean mitotic rate of 5 mitotic figures per 10 HPFs. Seven tumors had notable tumor cell necrosis. Diffuse p53 and p16 staining was seen in 2 cases. Immunoreactivity with antibodies to ER and PR was seen in all cases and was variable in degree. Ki67 staining indices varied and 9 cases showed <5% staining. Two cases recurred. Both had infarct-type necrosis, ki67 indices of <5%, and diffuse staining with antibodies to p16 and p53.

Conclusions: Most tumors designated as STUMPs behave in a benign fashion. The only 2 tumors that recurred in this study were diffusely immunoreactive with antibodies to p16 and p53.

Reviewer's Comments: This is an interesting study that reviews a large series of uterine tumors designated as STUMPs. Pathologists who review uterine specimens may wish to read this article to see the heterogeneity of tumors that may be classified as such. (Reviewer-Edward B. Stelow, MD).

print tag: () Refer to original journal article.
Identify Responders to Anti-EGFR Cancer Therapy via FISH

EGFR Antagonists in Cancer Treatment.
Ciardiello F, Tortora G:

Identification and selection of patients is a challenge for the clinical use of anti-EGFR agents for cancer treatment. Responses to anti-EGFR agents are seen in only 10% to 20% of patients.

Objective: To review the current roles of anti-epidermal growth factor receptor (anti-EGFR) therapies, including monoclonal antibody and small-molecule tyrosine kinase inhibitors (TKI).

Results: Some, but not all, patients with metastatic non-small-cell lung cancer, head and neck squamous cell carcinoma, colorectal cancer, and pancreatic cancer are candidates for anti-EGFR drugs. Erlotinib (Tarceva), a small-molecule TKI, is approved as second- or third-line treatment for advanced chemotherapy-resistant non-small-cell lung cancer. Another TKI, gefitinib (Iressa), was initially FDA-approved for metastatic non-small-cell lung cancer after failure with conventional platinum-based and docetaxel-based chemotherapies. However, when a large clinical trial failed to confirm benefit, gefitinib was restricted to investigational use in the United States and Europe. Whether anti-EGFR monoclonal antibody therapy using cetuximab will have role in non-small-cell lung cancer therapy remains to be proven. Cetuximab is FDA-approved, either alone or combined with irinotecan, for treatment of metastatic colorectal cancer, but it is approved by the European Medicines Evaluation Agency (EMEA) only when combined with irinotecan. Cetuximab is also FDA- and EMEA-approved when combined with radiotherapy for locally advanced head and neck squamous cell carcinoma. Another anti-EGFR monoclonal antibody, panitumumab, is also FDA-approved for metastatic colorectal cancer. Erlotinib in combination with gemcitabine is FDA- and EMEA-approved for pancreatic carcinoma. Asian race, nonsmoking history, and adenocarcinoma subtype predict non-small-cell lung cancer response to TKIs. In general, a cutaneous toxic reaction to anti-EGFR therapies predicts tumor response. EGFR gene mutations are associated with Asian race, nonsmoking history, and adenocarcinoma histology. The presence of mutations predicts non-small-cell lung cancer response to TKI. Increased EGFR copy number detected by FISH predicts non-small-cell lung cancer response to TKI and predicts colorectal cancer response to anti-EGFR monoclonal antibodies. Activating $K$-RAS mutations, most often occurring in codons 12 and 13 of exon 2, predict resistance to EGFR TKI in non-small-cell lung cancer and anti-EGFR antibody therapy in colorectal cancer. Finally, acquired exon 20 $K$-RAS mutations are associated with acquired resistance to TKI in non-small-cell lung cancer.

Reviewer's Comments: Identification and selection of the <20% of cancer patients who will respond to targeted EGFR therapies is facilitated by FISH testing for EGFR copy number and testing for activating $K$-RAS mutations. (Reviewer-Guy E. Nichols, MD).

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miRNAs Are Candidate Molecules for Cancer Diagnosis, Therapy

MiRNAs and Cancer.

Visone R, Croce CM:

Am J Pathol; 2009; 174 (April): 1131-1138

Small noncoding microRNA molecules that regulate protein translation are candidate molecules for both cancer diagnosis and targeted cancer therapy.

Objective: To review the biology as well as the prospective diagnostic and therapeutic utility of microRNAs (miRNAs) in cancer therapy.

Results: MicroRNAs (miRNAs) are small noncoding RNA molecules ranging from 17 to 27 nucleotides that regulate protein translation. Ribonucleoprotein-miRNA complexes bind to coding mRNAs, leading to either mRNA degradation or inhibition of translation. The expression and function of miRNA can be altered by (1) chromosomal instability that alters miRNA copy number; (2) DNA methylation of CpG islands found in roughly 50% of miRNA genes; (3) mutations in miRNA genes or adjacent regions; or (4) altered miRNA processing, such as diminished Drosha RNase III activity that is required for processing of precursor molecules (pri-miRNA). Expression profiles for miRNA appear to be altered in all neoplasms investigated to this date, suggesting a role for miRNA in tumorigenesis. Among numerous individual examples, overexpression of miR-155 is associated with several lymphomas and pre-lymphomatous proliferation in a transgenic murine animal model; mir-15a/16 plays a role in decreasing cyclin D1 and other cyclin proteins; and miR-21 is implicated in potentiating invasiveness and metastatic potential. Expression profiles for miRNA have shown distinct association with lineage of malignant tumor differentiation, and a miRNA-based tissue array has proven superior to mRNA expression profiling for predicting primary sites of metastatic tumors of unknown origin. Replacement miRNA therapies are being investigated for proliferations associated with decreased miRNA. In animal models, an antisense oligonucleotide inhibits activity of the hepatic miRNA miR-122.

Reviewer's Comments: Evidence suggesting specific roles for miRNAs in carcinogenesis still must be validated, possibly in genetically engineered animal model systems that lack or overexpress candidate miRNA molecules. Even more important, investigation into diagnostic and therapeutic applications of miRNA biochemistry requires extensive validation. Anti-miRNA drugs seem likely to have a wide range of associated effects, some of which may be impossible to predict. (Reviewer-Guy E. Nichols, MD).

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# Cytologic TLI Pattern in HT Representative of Clinical Picture

Cytologic TLI Pattern in HT Representative of Clinical Picture

## Cytologic, Flow Cytometry, and Molecular Assessment of Lymphoid Infiltrate in Fine-Needle Cytology Samples of Hashimoto Thyroiditis.

Zeppa P, Cozzolino I, et al:

*Cancer Cytopathol; 2009; 117 (June 25): 174-184*

| Thyroid lymphocytic infiltrates in Hashimoto thyroiditis can show small light chain imbalances and are not associated with immunoglobulin heavy chain rearrangements. |

## Background:

Hashimoto thyroiditis (HT) is the most common autoimmune condition of the thyroid, characterized by replacement of the thyroid parenchyma by lymphoid infiltrates (TLIs) and germinal center formation. Rarely, non-Hodgkin lymphoma (NHL) can arise in thyroid in this setting. Flow cytometry and immunoglobulin heavy chain gene rearrangements (IgH) are some of the most powerful tools in the diagnosis of B-cell lymphoma.

## Objective:

To describe the flow cytometry and IgH characteristics of non-neoplastic TLIs found in HT.

## Methods:

34 patients with a clinical diagnosis of HT and adequate thyroid fine-needle aspiration material for slide preparation and flow cytometry were included. Special follow-up on these patients was performed to ensure that lymphoma of the thyroid did not develop within the next 3 years. Aspirate smears were reviewed to confirm a diagnosis of HT according with standard cytologic criteria. Cases were subdivided into lymphocytic smears (isolated or intermingled small lymphocytes, plasma cells, and histiocytes), lymph node-like smears (mixture of small and large lymphocytes, germinal center cells, and plasma cells), or mixed smears (showing both patterns). Flow cytometry was performed on material from a dedicated pass. The kappa /lambda ratio was considered imbalanced if >=4.0. DNA was extracted from a dedicated pass in 4 cases for IgH PCR.

## Results:

Cytologic examination demonstrated 12 cases of HT with a lymphocytic pattern, 14 with a lymph node-like pattern, and 8 with a mixed pattern. Flow cytometry showed 2 main cell populations; CD3+CD5+ T-cells and CD19+ B-cells. The CD19+ B-cells comprised >20% of cells in 22 of 34 cases. The lymph node-like pattern was significantly associated with an abundance of CD19+ B-cells. The kappa /lambda ratio was >=4.0 in 5 cases (range, 4.0 to 5.0). Three of the 5 cases with unbalanced light chain ratios had limited B-cells, while the additional 2 cases had abundant B-cells. IgH PCR was attempted on the 5 cases with unbalanced light chain ratios: 1 case had insufficient DNA (with limited B-cells), and the remaining 4 cases were negative for a clonal IgH gene product (2 with limited B-cells and 2 with abundant B-cells).

## Conclusions:

Thyroid lymphocytic infiltrates in patients with HT show a mixture of B-cells and T-cells. Small light chain imbalances can be seen and are not associated with immunoglobulin heavy chain rearrangements. Fine-needle aspiration coupled with FC can help in distinguishing florid TLIs in HT from the development of a NHL.

## Reviewer's Comments:

The most worrisome scenario is a patient with abundant B-cells and an imbalanced ratio on thyroid FNA. However, in this study, all such cases were negative for IgH clonality. Nonetheless, in practice, patients with this finding should be followed up closely to exclude early NHL. (Reviewer-Deborah J. Chute, MD).

**print tag:** () Refer to original journal article.
SISH, CISH Show Good Correlation for HER2 Amplification

Bright-Field In Situ Hybridization for HER2 Gene Amplification in Breast Cancer Using Tissue Microarrays: Correlation Between Chromogenic (CISH) and Automated Silver-Enhanced (SISH) Methods With Patient Outcome.

Francis GD, Jones MA, et al: Diagn Mol Pathol; 2009; 18 (June): 88-95

There is a high level of concordance between CISH and SISH as methods to detect HER2 gene amplification.

Background: HER2 gene amplification is an important predictor of survival in breast cancer patients, as well as a therapeutic target for trastuzumab. The current gold standard for assessment of HER2 status is FISH, but this method is expensive, technically demanding, does not produce an archival slide, and requires special equipment. An alternative assessment method using bright field techniques is chromogenic in situ hybridization (CISH), which has shown very good correlation with FISH. A new method recently described uses an automated silver-enhanced in situ hybridization (SISH) to assess HER2 amplification.

Objective: To evaluate the performance of CISH and SISH to assess HER2 amplification in breast cancer, and to correlate their results with patient outcome.

Methods: A tissue microarray was constructed from 589 patients with breast carcinoma using dual cores for each patient and was analyzed with HER2 CISH and HER2 SISH. Gene amplification was assessed for CISH and SISH as follows: 1 to 2.5 copies/nucleus = diploid, >2.5 to 4 copies/nucleus = polysomy, >4 to 6 copies/nucleus = equivocal, 6 to 10 copies/nucleus = low level amplification, and >10 copies/nucleus = high level amplification. A sequential slide was used for detection of chromosome 17 (CHR17) with SISH, and a HER2/CHR17 ratio was calculated (<1.8 nonamplified, 1.8 to 2.2 equivocal, >2.2 amplified).

Results: 337 tissue cores and 230 patient samples had results from both methods. CISH HER2 analysis identified 25 patients (11%) with high amplification, 9 (4%) with low amplification, and 6 (3%) with equivocal results. SISH HER2 analysis identified 28 patients (12%) with high amplification, 7 (3%) with low amplification, and 3 (1%) with equivocal results. The SISH HER2/CHR17 ratio identified 29 patients (13%) with high amplification, 4 (2%) with low amplification, and 3 (1%) with equivocal amplification. There was agreement between CISH and SISH results in 96% of patients. A single case showed low amplification by both CISH and single-probe SISH, but had high-level polysomy on dual-probe SISH. When correlated with patient outcome, both CISH and SISH showed nearly identical survival curves.

Conclusions: There is a high level of correlation between CISH and SISH methods of detecting HER2 amplification. Patient survival prediction was nearly identical based on the CISH and SISH HER2 results.

Reviewer's Comments: SISH offers several advantages over CISH, including a shorter incubation period and automation, and it shows similar rates of HER2 amplification detection. However, it currently is only available on a Ventana platform, which may limit use in some laboratories. (Reviewer-Deborah J. Chute, MD).

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To Differentiate Spitz Nevus From Melanoma, Try p16

p16 Expression Differentiates Between Desmoplastic Spitz Nevus and Desmoplastic Melanoma.
Hilliard NJ, Krahl D, Sellheyer K:
J Cutan Pathol; 2009, 36 (July): 753-759

p16 expression is usually positive in desmoplastic Spitz nevi and negative to only weakly positive in desmoplastic melanoma.

Background: The differential diagnosis among spindle cell melanocytic neoplasms may be quite challenging. An example of such a diagnostic dilemma involves distinguishing a desmoplastic Spitz nevus from a desmoplastic melanoma. In >50% of sporadic cases of melanoma, p16 (a cell cycle regulatory protein) is lost. Although p16 expression by immunohistochemistry (IHC) has not shown a linear correlation with Breslow thickness in cases of melanoma, the loss of p16 expression has been associated with deeply invasive and metastatic melanomas.

Objective: To characterize the expression of p16 by IHC in cases of desmoplastic melanoma and desmoplastic Spitz nevi.

Methods: 15 desmoplastic Spitz nevi and 11 desmoplastic melanomas were retrieved. Each case was examined with HE staining and with IHC stains for p16, nestin, HMB45, and S100. The percent of positive tumor cell staining and the intensity of staining were recorded.

Results: All 15 Spitz nevi (100%) were positive for p16, showing moderate to strong expression intensity. In contrast, 2 desmoplastic melanoma cases (18%) showed only weak p16 positivity. Desmoplastic Spitz nevi and desmoplastic melanoma tumor cells showed similar tumor cell staining percentages with nestin, HMB45, and S-100.

Conclusions: p16 expression by IHC may aid in the differential diagnosis of desmoplastic Spitz nevus versus desmoplastic melanoma. Diffuse moderate to strong p16 expression is seen in desmoplastic Spitz nevus, while only focal weak staining is seen in desmoplastic melanomas.

Reviewer's Comments: Since more common markers of melanocytic differentiation are not very helpful in this particular differential diagnostic problem (HMB45, MART-1, tyrosinase, etc), p16 may be helpful in this setting. (Reviewer-T. David Bourne, MD).

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Gene Chip Technology Helps Predict Meningioma Outcome

Gene Expression Profiles of Meningiomas Are Associated With Tumor Cytogenetics and Patient Outcome.


Gene expression profiling may have more power to predict clinical outcome than histologic classification alone.

**Background:** Although most World Health Organization (WHO) grade I meningiomas are considered benign neoplasms that are often amenable to surgical treatment, recurrence is experienced by a significant percentage of patients. Recurrence is an even greater problem in cases of atypical or anaplastic meningiomas.

**Objective:** To analyze the gene expression patterns representative of clinically relevant cytogenetic subgroups of meningiomas.

**Methods:** 55 men and 117 women with a diagnosis of meningioma were included in the study. Of these patients, 95% underwent resection, and the tumors were classified using current WHO diagnostic criteria (grade I, 91%; grade II, 8% [atypical meningiomas], and grade III, 1% [anaplastic]). Forty-seven of these cases were prospectively selected for gene expression profiling (GEP) analysis using oligonucleotide microarrays, given that these tumors had known cytogenetic profiles defined by interphase fluorescence in situ hybridization (iFISH). iFISH was performed to detect numerical chromosomal abnormalities using probes for chromosomes 1, 7, 9, 10, 11, 14, 15, 17, 18, 22, X, and Y. GEP analysis was performed using the human genome Affymetrix(R) U133A-Chip. The microarray data were analyzed to determine whether tumor histology, grade, and associated cytogenetic findings were associated with a pattern of gene expression.

**Results:** Six meningioma subgroups were identified based on cytogenetic classification: diploid tumors; tumors with monosomy 22/22q- alone; tumors with del(1p36) alone; tumors with isolated sex chromosome loss; tumors with complex karyotypes without del(1p36) and loss of chromosome 14; and tumors with complex karyotypes with del(1p36) and/or chromosome 14 loss. Analysis of recurrence-free survival (RFS) showed significant differences among these various cytogenetic subgroups: tumors showing del(1p36) alone showed the highest percent RFS while tumors harboring complex karyotypes with del(1p36) and/or 14q loss showed the lowest percent RFS. The cytogenetic subgroup, used as an indirect surrogate for patient outcome, could be predicted with an error rate of <1% using GEP. No such association was found using tumor histology alone.

**Conclusions:** There is a clear association between the cytogenetic profiles of clinically distinct meningioma subgroups and patient outcome. This association can be predicted by GEP analysis. Such predictive power is lacking when histology alone is used.

**Reviewer's Comments:** The authors report interesting results supporting the less-than-optimal utility of the current WHO classification system for predicting clinical outcome among the various histologic subtypes of meningiomas. As the authors suggest, future use of ancillary cytogenetic testing, especially among the large and commonly encountered group of grade I tumors, will likely add more meaningful predictive and prognostic information than histologic subtyping alone. (Reviewer-T. David Bourne, MD).

**print tag:** () Refer to original journal article.
**MTA1 Predicts Malignancy in Pancreatic Endocrine Tumors**

*Immunohistochemical and Clinicopathological Correlation of the Metastasis-Associated Gene 1 (MTA1) Expression in Benign and Malignant Pancreatic Endocrine Tumors.*


Strong *MTA1* expression in pancreatic endocrine tumors corresponds to other clinicopathologic features that suggest malignant potential.

**Background:** Pancreatic endocrine tumors (PET) are typically well-differentiated with an indolent clinical course, but malignant potential cannot be predicted by histomorphologic features alone. The metastatic-associated protein 1 (*MTA1*) is a potential downstream effector of c-myc and is known to promote migration and invasion of carcinoma cells. The expression of *MTA1* is associated with metastatic disease in several carcinomas, such as breast, prostate, esophagus, and stomach. Others have shown expression of *MTA1* in neuroendocrine tissues, but its role in metastatic neuroendocrine tumors has not been explored.

**Objective:** To determine whether the expression of *MTA1* predicts the metastatic potential of PET.

**Methods:** 27 patients with available tissue from sporadic PET were included in the study. Each tumor was classified according to current World Health Organization (WHO) criteria, including: well-differentiated endocrine tumor with benign behavior (WHO 1.1) or with uncertain behavior (WHO 1.2), well-differentiated endocrine carcinoma (WHO 2.0), and poorly differentiated endocrine carcinoma (WHO 3.0). Immunostaining was performed for Ki-67 and *MTA1*. Scoring of nuclear *MTA1* staining was as follows: 1=negative, 2=weak, 3=moderate, and 4=strong.

**Results:** The mean patient age was 57 years at presentation (range, 28 to 86 years). The tumors ranged from 0.1 to 18 cm, with an average diameter of 4.5 cm. The average *MTA1* expression was 3.8 in tumors classified as malignant (n=17) compared to 2.9 in those classified as benign (n=10). This difference was significant. Statistical significance of the *MTA1* score was also found across WHO class, tumor size, and mitotic rate. Only borderline significance of *MTA1* was detected with the presence or absence of local invasion (*P* =0.062).

**Conclusions:** *MTA1* expression is significantly stronger in PET with features associated with or suggestive of malignant or metastatic potential.

**Reviewer's Comments:** The authors used immunohistochemistry for *MTA1* in PET, a putative biomarker of metastatic potential in various carcinomas. They found a significantly higher level of expression, corresponding to WHO classifications and other features that would be associated with potential malignancy. (Reviewer-Mary T. Galgano, MD).

**print tag:** () Refer to original journal article.
IGF2 Has Prognostic Significance in Stromal Tumors

Expression of Insulin-Like Growth Factor 2 in Mesenchymal Neoplasms.

Steigen SE, Schaeffer DF, et al: Mod Pathol; 2009; 22 (July): 914-921

IGF2 is expressed in a subset of gastrointestinal stromal tumors, solitary fibrous tumors, malignant peripheral nerve sheath tumors, and undifferentiated pleomorphic sarcomas, among others.

Background: The insulin-like growth factors (IGF) signaling system regulates growth and development in various tissues under normal physiological conditions, especially in the prenatal period. However, we have an emerging understating of the role of these factors in oncogenesis and tumor progression. IGF circulates in the bloodstream and interacts with receptors that signal through MAP kinase and PI3 pathways. High circulating levels of IGF have been associated with colon, breast and prostate cancers, and mesenchymal tumors have been shown to produce a prohormone form of IGF2. IGF2 has even been shown to have very high expression in gastrointestinal stromal tumors (GISTs) and synovial sarcomas, but confirmation at the protein level should be performed.

Objective: To survey IGF2 protein expression within a variety of mesenchymal tumors.

Methods: Tissue microarrays representing a variety of mesenchymal tumors (n=1288) were utilized for immunohistochemistry for IGF2. Tissue cores with >50 tumor cells were scored semiquantitatively for membranous or cytoplasmic staining. Results for the GISTs (n=449) were correlated to the clinicopathologic features that had been established on whole tissue sections.

Results: 39% of the GISTs were positive for IGF2, with high expression corresponding to significantly worse clinical outcome than those with low expression. Solitary fibrous tumors had the highest expression profile (80% of tumors positive). However, malignant peripheral nerve sheath tumors (46%), synovial sarcomas (43%), myxoid liposarcomas (39%), chondrosarcoma (33%), MFH (32%), Ewings sarcoma (27%), tenosynovial giant cell tumor (24%) also had significant staining.

Conclusions: Protein expression of IGF2 appears consistent with gene expression arrays and can be observed in a variety of mesenchymal neoplasms. High levels of expression in GISTs are associated with a worse clinical outcome.

Reviewer's Comments: The authors find high levels of IGF2 protein expression in a variety of mesenchymal tumors, especially in solitary fibrous tumors and GISTs, both of which have been noted to present with hypoglycemic episodes. While not a diagnostically useful stain, the expression of IGF2 may have prognostic and therapeutic implications. (Reviewer-Mary T. Galgano, MD).

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Protocols Crucial for Urgent Reporting of Critical Values

Critical Diagnoses in Surgical Pathology: A Retrospective Single-Institution Study to Monitor Guidelines for Communication of Urgent Results.


Laboratories should develop and monitor protocols for the reporting of critical values in surgical pathology.

Background: Critical values are considered those which connote derangements that may be life-threatening if not acted on immediately. Currently, clinical lab results considered critical are required to be reported to the individual responsible for the test results. This practice, however, is not as well established in anatomic pathology. Recently, organizations, such as the Association of Directors of Anatomic and Surgical Pathology, endorsed the concept of critical values in surgical pathology and published a guideline listing a number of critical values.

Objective: This manuscript discusses the experiences of a single academic institution after their establishment of policy and guidelines regarding the timely communication and reporting of unexpected surgical pathology findings.

Methods: After the establishment of policy and guidelines regarding the timely communication and reporting of unexpected surgical pathology findings, a retrospective review of two 6-month periods (the first in 2006 and the second in 2007) of all surgical pathology cases was conducted. All reports were searched using a number of means to identify those with documented notifications and diagnoses identified as critical values.

Results: Slightly more specimens were reviewed during the second study period. Records indicated urgent physician notification in 3.2% of cases in 2006 and in 3.7% of cases in 2007. About 20% of the cases with immediate physician notification were done because of established critical values, mostly because of immediate clinical consequences, although both unexpected and discrepant findings and life-threatening infections prompted notification. Most of the other biopsies that had urgent physician notification were renal and cardiac biopsies. Most physicians were contacted within 24 hours of submitting the specimens. Documentation of the relaying of critical information improved from the 2006 to the 2007 study period.

Conclusions: The establishment of guidelines for the reporting of critical values is essential for surgical pathology practices. Documentation of notification and the monitoring of that notification are necessary to optimize patient care.

Reviewer’s Comments: This manuscript nicely describes a single institution’s experience with critical values in surgical pathology. Most institutions should have adopted some list of critical values for their practice by now. (Reviewer-Edward B. Stelow, MD).

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Fundic Gland Polyps Most Common Gastric Polyp Seen in US

The Current Spectrum of Gastric Polyps: A 1-Year National Study of Over 120,000 Patients.

Fundic gland polyps are by far the most common type of gastric polyp encountered in the United States. This may be due to the widespread use of proton pump inhibitors.

Background: The older literature reports that gastric polyps can be found in 3% to 5% of patients who undergo upper endoscopy. Also, about 60% were estimated to be hyperplastic polyps, and 30% were fundic gland polyps. Since these earlier studies, the indications for upper endoscopy have increased, and many patients are now treated with proton pump inhibition. Furthermore, effective treatment is now used against Helicobacter pylori infection.

Objective: To review the current prevalence of the various types of gastric polyps seen by a large specialized gastrointestinal laboratory.

Methods: A single specialized laboratory's surgical pathology files were reviewed for all gastric polyps seen within 1 year. The type and number of polyps were recorded, as were any concomitant gastric diseases.

Results: During this study, >120,000 patients underwent upper endoscopy. Nearly 80,000 patients underwent gastric biopsy or biopsies. Nearly 8,000 specimens were described as a nodule, mass, or polyp. Of these, 84% had histologic findings that correlated with the endoscopic findings. Fundic gland polyps were by far the most common type of polyp detected and accounted for 77% of all polyps. Very rarely, low-grade dysplasia was also noted in these polyps. Hyperplastic polyps or "polypoid foveolar hyperplasia" accounted for 17% of all polyps, and dysplasia was seen in <1% of these cases. Malignant tumors accounted for about 2% of lesions, and a little more than half of these were adenocarcinomas. Gastric adenomas and carcinoid tumors each represented <1% of all polyps. Nearly 25% of patients with fundic gland polyps had chronic inactive inflammation, and 18% had changes of reactive gastropathy. Nearly 20% of patients with hyperplastic polyps had chronic, inactive inflammation, and 19% had changes of reactive gastropathy. Thirteen percent of these polyps, however, were associated with intestinal metaplasia, and 6% were associated with H pylori infection.

Conclusions: Fundic gland polyps are now by far the most common type of gastric polyp encountered in the United States. The authors speculate that is likely due to the widespread use of proton pump inhibitors.

Reviewer's Comments: This is a very large study of gastric polyps seen in the United States. It is interesting to note how seldom gastric adenomas are seen. (Reviewer-Edward B. Stelow, MD).

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