Desmoplastic Melanoma -- Identifying Type May Help With Prognosis

Prognostic Factors in Cutaneous Desmoplastic Melanoma: A Study of 252 Patients.

Murali R, Shaw HM, et al:

Cancer 2010; June 8 (): epub ahead of print

Pure desmoplastic melanomas were significantly associated with longer time to disease recurrence than combined desmoplastic melanomas.

Background: Desmoplastic melanoma (DM) is characterized by malignant spindle cells embedded in a fibrocollagenous stroma. DMs are uncommon and most often occur in the head and neck region. However, criteria defining how much desmoplasia is required to diagnose DM are still unclear. Previous studies have shown that patients with DMs present less frequently with sentinal lymph node (SLN) metastasis than patients with conventional melanoma.

Objective: To evaluate the prognostic value of various clinicopathologic factors in patients with DM, including extent of desmoplasia.

Participants: 252 patients with DM who underwent SLN biopsy.

Methods: Patients with a diagnosis of primary cutaneous melanoma which was at least partially desmoplastic were included in the study. All patients underwent SLN biopsy and complete excision. Melanomas were classified as pure DM if ≥90% of the invasive tumor was desmoplastic and as combined DM if <90% of the invasive tumor was desmoplastic. Clinical and pathologic parameters, as well as follow-up data, were obtained from the pathologic report and medical records.

Results: Mean patient age was 60.5 years, and the most common site was head and neck. Of tumors, 123 (49%) were pure DM and 129 (51%) were combined DM. Pure DMs were significantly associated with location in the head and neck, thicker depth of invasion, and neurotropism. SLN biopsies were positive in 7% of patients. SLN-positive patients were significantly associated with increasing tumor thickness. There was a trend for SLN metastasis to be less frequent in patients with pure DM-type tumors (5%) compared to combined DM-type tumors (8.5%). On multivariate analysis, increasing tumor thickness and positive SLN status were independent predictors of shorter disease-free survival. Combined DM type, ulceration, and positive SLN status were independently associated with shorter time to locoregional recurrence.

Conclusions: This study confirms the reduced rate of SLN metastasis in patients with DM. In addition, type of DM was significantly and independently associated with length of time to recurrence. The presence of a desmoplastic component in malignant melanomas should be identified and reported, as well as the type (pure or combined).

Reviewer's Comments: This is the largest study to date which examines patients with DM and SLN biopsy. It is interesting that although pure DMs were more commonly neurotrophic, they were less likely to develop lymph node metastasis and had a longer period to locoregional recurrence. This supports the notion that reporting of DM type may be helpful in predicting patient outcome. (Reviewer-Deborah J. Chute, MD).

Keywords: Desmoplastic Melanoma, Diagnosis, Melanoma, Pathology, Prognosis, Sentinel Lymph Node Biopsy, Skin

Print Tag: Refer to original journal article

Sessile Serrated Adenomas Pose Greater Risk Than Hyperplastic

Longitudinal Outcome Study of Sessile Serrated Adenomas of the Colorectum: An Increased Risk for Subsequent Right-Sided Colorectal Carcinoma.

Lu F-I, van Niekerk DW, et al:

Am J Surg Pathol 2010; 34 (July): 927-934

Patients with sessile serrated adenomas are more likely to develop adenocarcinoma than those with hyperplastic polyps.

Background: Previously, colorectal polyps were primarily diagnosed as adenomatous or hyperplastic and the teaching was that adenomatous polyps were neoplastic and premalignant. Over the past 20 years, it has come to be recognized that some polyps demonstrating a serrated architecture similar to conventional hyperplastic polyps are neoplastic and precursors to colorectal adenocarcinoma. These polyps are often devoid of conventional features of dysplasia and instead show an atypical architecture frequently with crypt dilation, lateralization, and branching. Adenocarcinomas associated with these tumors more frequently demonstrate high levels of microsatellite instability compared to those tumors that develop out of traditional adenomatous polyps. Currently, it is recommended that these polyps, most often referred to as "sessile serrated adenomas," be followed and treated as conventional adenomatous polyps.

Objective: To investigate the natural history of a series of patients with sessile serrated adenomas.

Participants: 81 polyps from 55 patients reclassified as sessile serrated adenomas.

Methods: All polyps diagnosed as "hyperplastic" at a single institution over a 21-period were reviewed. These cases had been diagnosed prior to use of the diagnosis "sessile serrated adenoma." Cases with crypt gland dilation, lateralization, serration, and branching were reclassified as sessile serrated adenomas. No traditional features of adenomatous change could be present. Matched control patients with hyperplastic polyps and adenomatous polyps were selected. Follow-up was pursued.

Results: Polyps represented approximately 6.0% of all polyps previously classified as hyperplastic and 1.5% of all polyps seen. Patients with sessile serrated adenomas were on average 63 years old and only slightly more likely to be male. Average follow-up for these patients was just over 7 years. Patients with sessile serrated adenomas were more likely to have a family history of colorectal adenocarcinomas. Nearly half of the sessile serrated adenomas were found in the distal colon. This was less frequent than both adenomatous and hyperplastic polyps. Of patients, 27% with sessile serrated adenomas eventually developed colorectal adenocarcinomas. This was compared to <2% of patients with hyperplastic or adenomatous polyps. They were also more likely to develop additional sessile serrated adenomas. Of adenocarcinomas that developed in patients who had had sessile serrated adenomas, 4 of 5 were found to be microsatellite unstable.

Conclusions: Patients with sessile serrated adenomas are at a much greater risk to develop colorectal adenocarcinomas compared to patients with hyperplastic polyps.

Reviewer's Comments: This manuscript highlights the importance of recognizing sessile serrated adenomas by histology. Pathologists should attempt to develop criteria for distinguishing these lesions from traditional hyperplastic polyps. (Reviewer-Edward B. Stelow, MD).

Keywords: Sessile Serrated Adenoma, Follow-Up, Adenocarcinoma, Hyperplastic Polyp

Print Tag: Refer to original journal article

Antibody Expiration Dates Not As Accurate As Once Thought

Antibody Expiration in the Context of Resource Limitation: What Is the Evidence Basis?

Savage EC, DeYoung BR:

Am J Clin Pathol 2010; 134 (July): 60-64

Expired antibodies often work as well as or even better than new reagent lots.

Background: The Clinical Laboratory Improvement Amendments (CLIA) act was passed in 1988 to develop quality standards for laboratories. Under CLIA, primary antibodies used in immunohistochemistry have been granted analyte status. This is addressed under the College of American Pathologists (CAP) Survey Checklist ANP 22432, prohibiting the use of primary antibodies beyond the manufacturer's printed expiration date. Problems occur with antibodies that are used infrequently. The fundamental issue is that many antibodies continue to work well long after the printed expiration date. Two previous studies addressed this and demonstrated no significant differences between new and expired reagents. The last study was over a decade ago, before the "analyte-specific-reagent era."

Objective: To investigate the quality of immunohistochemical results among primary antibodies and their expired counterparts.

Methods: 26 antibodies and their expired counterparts (average expiration interval of 13 months) were selected. Each antibody was used to stain consecutive formalin-fixed, paraffin-embedded tissue sections using standard immunohistochemical techniques. These antibodies encompassed most of the common antibodies ordered in routine clinical use, such as B72.3, calretinin, BRST-2, HBME, vimentin, etc. Slides were then assessed for staining intensity (1+ to 3+) and percentage of positive cells.

Results: Expired reagents worked as well as or better than the new antibodies for 23 antibodies tested. Regarding staining intensity, 22 showed no difference, 3 showed increased intensity with the expired reagent, and only with CD21 did the non-expired lot show increased intensity (3+ vs 2+). Regarding percent of cell positivity, 22 antibodies showed no difference, 1 (antiprolactin) showed increased numbers of cells staining with the expired lot, and 3 (HBME, p53, and TSH) demonstrated increased cell positivity with the non-expired lot.

Conclusions: CLIA places primary antibodies under the rubric of analyte-specific reagents, leading to the restrictions imposed by the CAP Survey Checklist ANP 22432. However, this study showed that 88% of expired antibody lots that were tested performed as well or better than new counterparts. Importantly, among differences with staining intensity or percentage of positivity, none differed by >1 increment, making these differences clinically negligible.

Reviewer's Comments: The current restrictions are understandable in light of the printed expiration dates; however, the authors raise 2 good points that should be addressed. First, manufacturers do not have time nor incentive to adequately evaluate the true life span of primary antibodies. Second, best practice dictates that positive and negative controls are run when using these antibodies and that should determine whether the test is valid or not. (Reviewer-William A. Kanner, MD).

Keywords: Antibodies, Immunohistochemistry, Expiration Date, Quality Assurance

Print Tag: Refer to original journal article

Identifying Muscle Layer Affected by Bladder Cancer Important for Prognosis

Smoothelin Immunohistochemistry Is a Useful Adjunct for Assessing Muscularis Propria Invasion in Bladder Carcinoma.

Bovio IM, Al-Quran SZ, et al:

Histopathology 2010; 56 (June): 951-956

Antibodies to smoothelin show moderate to strong staining of the muscularis propria in bladder biopsy and cystectomy specimens, allowing distinction from muscularis mucosae in most cases.

Background: Bladder cancer continues to rank among the top 10 causes of cancer-related deaths each year in the United States. More than 68,000 Americans were diagnosed with bladder carcinoma in 2008 alone. Approximately 80% of bladder cancers do not invade the muscle layer of the bladder wall at the time of presentation. The remaining 20% of tumors, however, either invade the more superficial muscularis mucosae (MM) or the deeper layers of the muscularis propria (MP). If limited to the MM of the lamina propria, patients are classified as having pathologic stage 1 (pT1) neoplasms. If there is invasion of the MP, however, the tumor is upstaged to at least a pathologic stage 2 (pT2), which is associated with a significantly worse prognosis (<50% 5-year survival). Making the distinction between MM and MP is sometimes challenging, especially if the specimen consists of transurethral biopsy (TURBT) fragments or if there is MM hypertrophy. While more commonly used smooth muscle markers appear to show similar staining in MM and MP, a more recently described antibody called smoothelin has been shown in one report to differentiate MM from MP. **Objective**: To prospectively evaluate the utility of smoothelin for the evaluation of MP in TURBT and cystectomy specimens. **Methods**: TURBT and cystectomy specimens over a 6-month period from patients with urothelial cancer were collected. Inclusion criteria for the use of smoothelin included the following: features on H&E-stained sections strongly suggestive of MP invasion, the question of whether MP was present in the specimen, or the question of whether the tumor was invading MP or hypertrophic MM. Paraffin sections were stained with anti-smoothelin mouse monoclonal antibodies, and expression intensity was then semiquantitatively evaluated as negative (0), weak (1+), moderate (2+), or strong (3+). **Results**: All cases except 2 showed strong (3+) moderate (2+) staining of MP. Only 1 case showed moderate (2+) staining of MM with smoothelin, while 97% showed weak or negative staining. This pattern of MM immunoreactivity mirrored that of blood vessel smooth muscle endothelium. Overall, smoothelin had a sensitivity of 92% for MP detection and a specificity of 97% for distinguishing MM from MP. **Conclusions**: Smoothelin is useful in helping to confirm the presence of MP, in helping to confirm MP invasion by tumor, and in distinguishing MP from MM in TURBT and cystectomy cases.

Reviewer's Comments: The findings of this article have significant practical utility given the frequency of TURBT biopsies and the clinical importance of accurately identifying the presence of MP and its invasion by tumor. Although the wispy smooth muscle fibers of the MM may show weak staining, the darker intensity of MP staining does seem to allow confident distinction of MP from MM. (Reviewer-T. David Bourne, MD).

Keywords: Bladder Cancer, Immunohistochemistry, Muscular Propria, Urinary Bladder Cancer

Print Tag: Refer to original journal article

Warty Carcinoma of the Penis Bears Careful Evaluation

Warty-Basaloid Carcinoma: Clinicopathological Features of a Distinctive Penile Neoplasm. Report of 45 Cases.

Chaux A, Tamboli P, et al:

Mod Pathol 2010; 23 (June): 896-904

A warty carcinoma of the penis should be evaluated for a basaloid component, as this may confer worse prognosis than a pure warty carcinoma.

Background: Squamous cell carcinomas of the penis have a wide range of histological subtypes that may have prognostic significance. Exophytic verruciform subtypes have a relatively good prognosis when compared to those without a papillomatous growth pattern, such as basaloid carcinomas. Occasionally, warty and basaloid features are noted in a single tumor, and on a large survey these were considered to represent a distinct subtype with a stronger association with human papillomavirus (HPV).

Objective: To present clinicopathologic features of a large series of warty-basaloid penile carcinomas.

Design: Retrospective review.

Participants: 45 patients with warty-basaloid carcinomas of the penis collected from 3 hospitals.

Methods: Clinical charts and pathologic material were reviewed and compared to previously reported squamous cell carcinomas of the penis, including warty, basaloid, and usual subtypes.

Results: Most patients presented in their 60's with a white-gray large irregular penile mass, but not all were exophytic. The biphasic tumors had variable proportions of warty and basaloid components, but most tumors (80%) had a greater proportion of the basaloid component. The most common pattern was a condylomatous tumor overlying the deep infiltrating basaloid tumor. Less often, the tumor was a non-papillomatous pattern of infiltrating nests with peripheral basaloid cells and central keratinizing cells with luminal necrotic and keratin debris. The rarest pattern was a papillomatous tumor composed of basaloid cells, clear cell koilocytosis and prominent fibrovascular cores. The tumors were generally high grade and 19 were associated with a precancerous lesion having either warty, basaloid, or combined features. Of patients, 21 had a groin dissection; 11 of these were positive. The metastases usually had the basaloid cytology with central necrosis and keratin debris. With follow-up in 21 patients, mean 5- and 10-year survival was estimated at 69 and 61%, respectively. Cancer-specific mortality was 33% with a mean survival of 2.8 years. In comparison to usual carcinomas, the warty-basaloid types were more frequently in the foreskin and were thicker tumors.

Conclusions: Warty-basaloid carcinomas of the penis are a distinct subtype that should be recognized for clinicopathologic features that tend to be more aggressive than pure warty carcinomas.

Reviewer's Comments: Not all of the clinicopathologic comparisons reached statistical significance, but warty-basaloid carcinomas tend to have more aggressive features than those that are pure warty. (Reviewer-Mary T. Galgano, MD).

Keywords: Squamous Cell Carcinoma, Warty-Basaloid Variant, Intraepithelial Neoplasia

Print Tag: Refer to original journal article

p16-Positivity Does Not Always Equal HPV-Positivity

p16INK4A Overexpression Is Frequently Detected in Tumour-Free Tonsil Tissue Without Association With HPV.

Klingenberg B, Hafkamp H, et al:

Histopathology 2010; 56 (June): 957-967

Upregulation of p16 can be detected in about one quarter of non-neoplastic tonsillar tissues, but its overexpression is likely caused by a mechanism other than HPV infection.

Background: In addition to alcohol and tobacco, infection of the epithelial tissues of the oropharynx by oncogenic human papillomavirus (HPV) is an increasingly recognized risk factor for the development of oropharyngeal squamous cell carcinoma (SCC). Biologically, HPV-positive tonsillar SCC differs from its HPV-negative counterpart. Among other features, the HPV-positive tumors usually show inactivation of various proteins associated with the p53 and retinoblastoma (Rb) tumor suppressor pathways, as well as upregulation of the cyclin-dependent kinases p16 and p21. In contrast, HPV-negative tumors usually show overexpression of p53, inactivation of p16, and epidermal growth factor receptor (EGFR) accumulation. Since p16 overexpression is linked with HPV positivity, it has been used as a surrogate marker of HPV infection. **Objectives**: To determine the prevalence of HPV in tumor-free tonsillar tissue and to assess the utility of p16 expression by immunohistochemistry as a surrogate marker of oncogenic HPV status.

Participants: 262 patients treated by tonsillectomy for various non-neoplastic conditions, including snoring and chronic tonsillitis. **Methods**: Tumor-free tonsillar tissue sections were collected from participants. DNA was isolated from each specimen and HPV-specific polymerase-chain-reaction (PCR) analysis was performed. Immunohistochemistry for p16 and fluorescence in situ hybridization (FISH) assays for HPV types 16 and 18 were also performed. Immunohistochemistry was semiquantitatively assessed by 3 observers. p16 overexpression was defined as moderate to strong nuclear and cytoplasmic staining. **Results**: p16 overexpression was present in 28% of samples. Positive staining was found within lymphoid germinal centers (52 of 187 cases) and in tonsillar crypt epithelium (49 of 177 cases). There was no immunoreactivity seen within the superficial squamous epithelium. PCR analysis identified HPV types 16 and 18 in 1% (2 of 195) of cases; cases which were negative for HPV by FISH. **Conclusions**: In some tonsillar tissues, upregulation of p16 is likely caused by a mechanism other than HPV infection.

Reviewer's Comments: This paper is interesting given its focus on the expression of p16 and the detection of HPV in non-neoplastic, rather than neoplastic, tonsillar tissue. Evidence from prior tumor studies certainly supports the strong association between p16 expression and oncogenic HPV-positivity in oropharyngeal carcinomas. In our own practice, we routinely use immunohistochemistry to assess p16 and EGFR status in oropharyngeal carcinomas, given the more favorable clinical outcomes seen in patients with p16 and/or HPV-positive tumors. (Reviewer-Stacey E. Mills, MD).

Keywords: Oropharyngeal Squamous Cell Carcinoma, p16, HPV

Print Tag: Refer to original journal article

Telecytopathology Comparably Accurate to On-Site Assessments

Telecytopathology for Immediate Evaluation of Fine-Needle Aspiration Specimens.

Alsharif M, Carlo-Demovich J, et al:

Cancer Cytopathol 2010; 118 (June 25): 119-126

Using telecytopathology for on-site adequacy assessments is as accurate as conventional on-site assessments.

Background: On-site evaluation of fine needle aspiration (FNA) biopsies is important to confirm sample adequacy and triage specimens for special studies. However, many cytology laboratories are hindered in providing this service because of the distance from multiple FNA procedure sites. Telepathology systems allow electronic transmission of microscopic images online for consultation. Dynamic telepathology provides live images which are viewed in real time, and has been used with encouraging results for frozen section consultation.

Objective: To report the authors' experience using dynamic telecytopathology for the evaluation of on-site adequacy assessments, and to compare it to the accuracy of conventional on-site evaluation by a pathologist.

Participants: 429 cases evaluated by telecytopathology and 363 cases evaluated by conventional on-site evaluation.

Methods: Air-dried Diff-Quik stained cytologic smears were prepared on-site by a cytopathology fellow or cytotechnologist. The telecytopathology system includes a digital camera, monitor, microscope, computer, and internet connection. The operator, typically the cytopathology fellow or senior resident, moved the slides on the stage to show diagnostic fields. A pathologist interpreted the cytologic images on their computer from their office in real time while communicating with the operator over the telephone. Preliminary assessment categories included: unsatisfactory, negative, adequate defer to final, atypical, neoplasm, suspicious, and positive for malignancy. Cases were considered discrepant with the final interpretation if there was a preliminary interpretation of negative and a final diagnosis of neoplasm, suspicious, or positive for malignancy. Rates of sample adequacy, deferral, and discrepant cases were compiled for 1 year prior to implementation of telecytopathology (conventional on-site adequacy assessment), and 1 year after implementation.

Results: In both groups, distribution of preliminary cytologic interpretations was similar. The final sample adequacy rate was 94% for telecytopathology and 98% for conventional assessment. The adequate-deferral rate was 13% for telecytopathology and 30% for conventional adequacy assessments. The discrepancy rate between preliminary and final diagnosis was 2% for telecytopathology and 3% for conventional on-site assessment, which was not significantly different. Most discrepant cases were due to a paucity of tumor cells on the initial Diff-Quik stained slides.

Conclusions: On-site evaluation of FNA specimens by telecytopathology provides comparable sample adequacy and preliminary interpretations as conventional on-site assessments. This technology can allow pathologists to provide on-site evaluations to remote locations while still using their time efficiently.

Reviewer's Comments: Prior studies have shown that dynamic telepathology systems are more accurate than static image systems, particularly for cytology. The on-site operator plays a critical role in the success of this system. The authors note that slightly more cases were discrepant using telecytopathology in the early part of the fellow's training year, mainly due to their inexperience in cytology and on-site evaluation. (Reviewer-Deborah J. Chute, MD).

Keywords: Fine-Needle Aspiration, Telecytopathology, Rapid On-Site Evaluation, Adequacy Assessment, Preliminary Diagnosis

Print Tag: Refer to original journal article

Immediate Surgery May Not Be Answer With Low-Risk Prostate Cancer

Outcomes in Localized Prostate Cancer: National Prostate Cancer Register of Sweden Follow-Up Study.

Stattin P, Holmberg E, et al:

J Natl Cancer Inst 2010; 102 (July 7): 950-958

Patients with low-risk prostatic adenocarcinoma have a low risk of death at 10 years when initially treated with surveillance only.

Background: The possible overtreatment of prostate cancer has been discussed extensively in the literature. Some have thus suggested that watchful waiting or active surveillance may be a possible options for patients found to have "low-risk" prostate cancer on biopsy. Recently, a randomized study showed a decreased death-rate from prostate cancer in patients undergoing screening, but also found that 48 patients had to undergo curative therapy to save a single life.

Objective: To calculate risk of death from prostate cancer based on clinicopathologic factors and treatments using a national register of patients with prostrate cancer.

Design: Retrospective cohort study.

Participants: 6849 patients with localized prostate cancer who were aged ≤70 years.

Methods: A national cancer registry that is estimated to collect 98% of solid cancer diagnoses was used. For prostate cancer, data recorded by the register included prostrate-specific antigen (PSA) level, TNM stage, tumor differentiation, and treatment. Only patients with Gleason score ≤7, T1 or T2 tumors without metastases, and with serum PSA levels of <20 ng/mL were included in this study. Treatment and follow-up data were obtained. Low-risk disease was considered T1 tumor with a Gleason score of ≤6 and a serum PSA of <10 ng/mL.

Results: 2686 of cases were considered to be low-risk. Approximately one third of patients underwent surveillance. Of patients, 40% with low-risk tumors underwent surveillance. One third of patients initially treated with surveillance eventually underwent curative therapy. Median follow-up was 8 years. Patients in the curative groups were in better health and had better survival than average for similar aged, non-cancer patients. The 10-year calculated mortality from prostate cancer was 3.6% in the group who underwent surveillance and 2.7% in patients who initially underwent curative therapy. For low-risk disease, the calculated 10-year mortality from prostate cancer was 2.4% for those undergoing surveillance and 0.7% for those initially treated with curative therapy.

Conclusions: Active surveillance may be a suitable option for some patients diagnosed with prostate cancer, especially for patients with low-risk disease. Relatively healthy individuals should know that they fare better at 10 years if they undergo prostatectomy.

Reviewer's Comments: Patients with low-risk prostate cancer who do not undergo immediate surgery have only about a 2% chance of dying from their cancers in 10 years. (Reviewer-Edward B. Stelow, MD).

Keywords: Adenocarcinoma, Therapy, Waiting, Outcome

Print Tag: Refer to original journal article

Counting Lymph Nodes Is Not Exact Science

To Count and How to Count, That Is The Question: Interobserver and Intraobserver Variability Among Pathologists in Lymph Node Counting.

Parkash V, Bifulco C, et al:

Am J Clin Pathol 2010; 134 (July): 42-49

Lymph node counting is not a straightforward task, and it is likely related to the non-standard definition of what is a "countable" lymph node.

Background: There is now pressure on both surgeons and pathologists to identify a minimum number of nodes for certain procedures. From the pathologist's perspective there are various procedures to dissect lymph nodes (LN), including palpation and various solutions to digest away fat and highlight possible node candidates. There are also different approaches to counting lymph nodes on microscopic slides (ie, only counting encapsulated collections of lymphocytes).

Objective: To assess whether there is standardization in LN dissection and counting at 2 institutions (academic center and large community hospital).

Methods: For assessing LN retrieval, a questionnaire was circulated to the 2 institutions. Residents and physician's assistants were the primary prosectors at the academic center and community hospital, respectively. For LN counting, 15 slides with gross descriptions were circulated among 10 pathologists (3 of which practiced at the academic center). The only instructions where to count the LNs on the slides. The slides where also recirculated at a second time point (2 to 6 weeks) to assess intraobserver variability. A follow-up questionnaire was then circulated and, finally, a roundtable discussion was conducted. Various statistical analyses were performed.

Results: Per self-report, the gross dissection of lymph nodes was relatively standardized at both institutions. Most large specimens were subjected to fat clearing with some variations. Smaller specimens had more variation, including dissection for lymph nodes versus just submitting the entire specimen without dissection. All dissectors submitted the entire node unless grossly involved by tumor. Regarding LN counts, among the 15 slides the total counts varied from 62 to 101 among pathologists and no single slide was found to have agreement by all reviewers. The 2 slides with the greatest interobserver variability had >10 lymphoid "fragments." Overall variability in LN counts was 11% and there was greater inter- versus intraobserver variability. Further statistical calculations demonstrated for this data set that there was a confidence interval of ±2.6 of the true number, thus indicating that pathologist counts could vary by up to 2.6 LNs. Half of pathologists did not consider a dispersed collection of lymphocytes without a capsule to be a LN. Half of pathologists also did not count minute LN structures (<1 mm), yet they were counted when tumor was present.

Conclusions: LN counting is not a straightforward task, and it is likely related to the non-standard definition of what is a "countable" lymph node. There are arguments for each philosophy behind what should be counted as a lymph node.

Reviewer's Comments: Lymph node counting, not just the number of positive nodes, is important and this article highlights the variability in counting LNs on the slide. I have also noticed this among the various pathologists I have worked with during my residency. (Reviewer-William A. Kanner, MD).

Keywords: Lymph Node Counting, Cancer Staging, Interobserver Variability, Intraobserver Variability

Print Tag: Refer to original journal article

Tumor Budding Is Significant in Esophageal Cancers

Tumour Budding and a Low Host Inflammatory Response Are Associated With a Poor Prognosis in Oesophageal and Gastro-Oesophageal Junction Cancers.

Brown M, Sillah K, et al:

Histopathology 2010; 56 (June): 893-899

The presence of significant tumor budding is an independent prognostic factor in patients with esophageal or gastroesophageal cancers.

Background: Tumor budding, which is seen along the invading edge of many tumors, consists of small tumor cell clusters or individual tumor cells with no evidence of differentiation. Prior studies have shown that prominent tumor budding in esophageal squamous cell carcinoma (SCC) and gastric adenocarcinoma is associated with a worse prognosis. In addition to budding, the degree of host inflammatory response has been identified as an independent prognostic feature of better survival in patients with esophageal SCC. **Objectives**: To assess prognostic significance of tumor budding and host inflammatory response in esophageal and gastroesophageal junction cancers.

Design: Retrospective study.

Participants: 356 patients who had transthoracic esophagectomy or esophagogastrectomy procedures for esophageal or gastroesophageal cancer. **Methods**: Patients with gastric carcinoma were excluded from the study. Of patients, 115 (32.3%) were also treated with neoadjuvant chemotherapy. From each case, the slide showing the deepest invasion was selected and examined for the presence of tumor budding and host inflammation. Tumor budding was strictly defined as a small group of <5 cells or a single cell at the advancing edge of an invading tumor without evidence of differentiation. Low-power examination was used to locate the area of greatest tumor budding. Then, using 20x magnification, the total number of buds in one field was counted and classified as <5 buds or ≥5 buds. Degree of host inflammation was assigned to 1 of 3 categories: sparse, moderate, or pronounced. Consensus scores were obtained for any scoring discrepancies. Survival data were collected from hospital records. **Results**: A median of 4 tumor buds was identified per case, with 172 cases having ≥5 buds (48.3%). By univariate and multivariate analysis, the presence of >5 buds tumor buds was associated with a poor prognosis (P =0.0001 for univariate; P =0.002 for multivariate). Degree of tumor budding retained significance even when tumors were separated into adenocarcinoma versus squamous cell carcinoma groups. Degree of host inflammation, in contrast, was only a significant factor for adenocarcinomas. **Conclusions**: The presence of significant tumor budding is an independent prognostic factor in patients with esophageal or gastroesophageal cancers, and this feature may be used to identify patients who might benefit from earlier follow-up and/or different neoadjuvant therapies.

Reviewer's Comments: As the authors point out, correctly identifying tumor budding as a pathological variable must be reproducible if such a feature is to be diagnostically useful. It does seem that the interobserver variation is good when tumor budding is carefully defined, as in this study. (Reviewer-T. David Bourne, MD).

Keywords: Esophageal Cancer, Tumor Budding, Host Inflammatory Response

Print Tag: Refer to original journal article

Chronic Chorioamnionitis Associated With Preterm Labor, ROM

The Frequency, Clinical Significance, and Pathological Features of Chronic Chorioamnionitis: A Lesion Associated With Spontaneous Preterm Birth.

Kim CJ, Romero R, et al:

Mod Pathol 2010; 23 (July): 1000-1011

Chronic chorioamnionitis with and without chronic villitis may be an immunological response associated with preterm labor and rupture of membranes.

Background: Acute chorioamnionitis is a common lesion to be noted in the placenta, especially after preterm labor and/or rupture of membranes (ROM). This is usually associated with a microbial invasion of the amniotic cavity with subsequent maternal and fetal neutrophils infiltrating the chorioamniotic membranes and umbilical cord. Severity is associated with adverse outcomes and may be causally related to preterm labor. However, normal delivery at term is associated with acute inflammatory features, possibly a normal physiological component of parturition. Chronic chorioamnionitis is characterized by lymphocytic infiltration of chorioamniotic membranes and chorionic plate. This has also been associated with preterm birth, but also with spontaneous abortion, intrauterine growth restriction, and chronic villitis of unknown etiology.

Objective: To evaluate clinical significance, frequency, and pathophysiology of chronic chorioamnionitis by comparing placentas from a variety of circumstances.

Participants: 700 placental tissues were collected from women.

Methods: Women included 100 not in labor at term without complications, 100 in labor at term, 100 in preterm labor without ROM, 100 in preterm prelabor ROM, 100 with pre-eclampsia at term, 100 with pre-term pre-eclampsia, and 100 with small-for-gestational-age neonates born at term. Amniocentesis samples were collected only from women with clinical indications necessitating the procedure (n=64). Samples were evaluated by immunohistochemistry, Enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) for T-cells and anti-angiogenic T-cell chemokines. Chronic chorioamnionitis was graded based on lymphocytes in the chorionic trophoblast layer or chorioamniotic connective tissues: 0- absent, 1- more than 2 foci or patchy inflammation, 2-diffuse inflammation. The stage was scored as: 1- limited to chorionic trophoblast layer sparing the chorioamniotic connective tissue and 2- infiltration into the chorioamniotic connective tissue.

Results: Preterm labor and preterm prelabor ROM cases had chronic chorioamnionitis in 34% and 39%, respectively. Pre-eclampsia at term, pre-eclampsia in pre-term, and small for gestational age were diagnosed as chronic chorioamnionitis in 23%, 16%, and 13%, respectively. Normal term not in labor and in labor was only 19% and 8%, respectively. Cases with chronic chorioamnionitis had higher levels of CXCL10 and higher mRNA expression of multiple chemokines. Chronic villitis was noted in over a third of the cases with chronic chorioamnionitis having preterm labor or ROM.

Conclusions: Chronic chorioamnionitis is commonly noted in preterm labor and preterm ROM and given the chemokine profile probably represents an immunological process.

Reviewer's Comments: The authors suggest the immunological process in the chorioamnioniotic membranes, and sometimes villi, is similar to transplant rejection or graft-versus-host disease. (Reviewer-Mary T. Galgano, MD).

Keywords: Chorioamnionitis, Amniotic Fluid, Pregnancy

Print Tag: Refer to original journal article

Pancreatic Endocrine Tumors -- Functioning Vs Non Functioning

Clinicopathological Features of Pancreatic Endocrine Tumors: A Prospective Multicenter Study in Italy of 297 Sporadic Cases.

Zerbi A, Falconi M, et al:

Am J Gastroenterol 2010; 105 (June): 1421-1429

Non-functioning pancreatic endocrine tumors are far more common than functioning pancreatic endocrine tumors in today's world.

Background: Pancreatic endocrine tumors represent <5% of pancreatic neoplasms. At one time, the majority of tumors were functioning -- a status based on clinical features not on immunohistochemical findings. Many have remarked that this no longer appears to be the case as tumors are frequently identified in asymptomatic patients with the increased use of radiologic procedures.

Objective/Design: To report the findings of a prospective, observational, multicenter study of newly diagnosed pancreatic endocrine tumors.

Participants: 297 patients with newly diagnosed pancreatic endocrine tumors seen over a 3-year period at 24 participating centers.

Methods: Cases diagnosed surgically or by biopsy were included. Cases were classified per the World Health Organization criteria as well-differentiated tumors, tumors with uncertain behavior, well-differentiated carcinomas, and poorly differentiated carcinomas. Ki-67 indices were recorded. Tumor-node-metastasis (TNM) status was recorded. Patients with MEN type 1 were excluded. Follow-up data were recorded.

Results: Nearly 80% of patients had undergone resection. Mean age was 59 years and there were slightly more women than men. Age did not differ significantly based on tumor classification. Approximately 25% of cases were considered functional. The majority of these were insulinomas and the second most common were gastrinomas. Functional tumors were more likely to be classified as benign (well-differentiated tumors). Of nonfunctioning tumors, 51% were asymptomatic. The other tumors most often presented with pain or weight-loss. Tumors were somewhat more common in the pancreatic tail. Mean size of tumors was 3.2 cm. Benign tumors were on average 1.5 cm, whereas carcinomas were on average 4 cm. Non-functioning tumors were on average nearly twice as large as functioning tumors. Of tumors, 31% were benign, 17% were considered tumors with uncertain behavior, 45% were well-differentiated carcinomas, and 8% were poorly differentiated carcinomas. Tumor type correlated with tumor size and stage. Serum chromogranin levels were increased in 42% of cases and levels were more likely to be increased in carcinomas. When liver or nodal metastases were present they were detected by CT in 96% and 64% of cases, respectively.

Conclusions: Non-functioning pancreatic endocrine tumors are far more common than functioning pancreatic endocrine tumors in today's world. Tumors now present with fewer metastases and at a smaller size than in the past.

Reviewer's Comments: Unfortunately, this manuscript does not actually break down risk of metastasis per tumor size and one is left to wonder how many small tumors metastasize. These data are essential for determining the management of the small tumors sometimes identified incidentally. (Reviewer-Edward B. Stelow, MD).

Keywords: Pancreatic Endocrine Tumors, Malignancy, Size, Functional Status

Print Tag: Refer to original journal article

Are There Large Variants of BDHs That Can Be Symptomatic?

Giant and Complicated Variants of Cystic Bile Duct Hamartomas of the Liver: MRI Findings and Pathological Correlations.

Martin DR, Kalb B, et al:

J Magn Reson Imaging 2010; 31 (April): 903-911

BDHs can undergo cystic enlargement and internal hemorrhage, which can present clinically with abdominal pain.

Objective: To determine if bile duct hamartomas (BDHs) can undergo cystic enlargement and internal hemorrhage and consequently produce clinical symptoms.

Design: Retrospective analysis.

Participants/Methods: This study was comprised of 15 patients, 11 females and 4 males, who had a surgical pathological diagnosis of a fibrocystic hepatic cyst and had undergone an abdominal MRI examination within a 1-month period. MR imaging was performed on a 1.5-T system and included single-shot T2-weighted, T1-weighted in-and-out of phase gradient echo, and T1-weighted 3D gradient echo multiphase contrast-enhanced fat-suppressed dynamic images obtained prior to and following gadolinium injection during arterial, venous, and delayed phases. Patients underwent cyst fenestration, hepatic resection, or both. The images and the surgical and histopathological reports were reviewed by 2 radiologists in conjunction with a hepatobiliary surgeon and pathologist.

Results: All of the cysts that had not been drained had well-defined, smooth, and lobulated margins, internal fluid content, thin septations, and a T2 hypointense rim. Complex internal fluid signal, which was interpreted as intracystic hemorrhage or proteinaceous material, was found in 10 of the 15 patients. Three of the 10 had a thick wall measuring >2 mm representing inflammation resulting from previous drainage. There were no enhancing intracystic vascularized soft tissue elements. There was no intra- or extrahepatic biliary dilatation in any of the patients. Ten of the 15 patients also had a few small simple cysts in the kidneys; however, there were no clinical or additional imaging findings to support a diagnosis of polycystic kidney disease. Fourteen of the 15 patients presented with abdominal pain that resolved with surgery. At pathology, all of the cysts had a smooth lining. A multilocular pattern along the cyst wall due to fibrous septations was seen in 12 patients. There were dilated bile ducts on the cyst walls in 7 patients, which are characteristic of BDHs. There were also BDHs in the remaining uninvolved portions of the liver. No ovarian type stroma was present as would be expected of a biliary neoplasm.

Conclusions: According to the authors, “BDH is a benign hepatic cystic lesion that may undergo cystic enlargement, internal hemorrhage, and clinically present with abdominal pain treatable by minimally invasive laparoscopic fenestration.”

Reviewer's Comments: The results of this study are helpful in demonstrating that bile duct hamartomas may enlarge and demonstrate complex internal fluid signal related to hemorrhage. Classically described as measuring <2 cm in size, this entity should be considered in the differential possibilities when encountered with a large cystic hepatic mass that does not contain soft tissue elements. One of the limitations of this study was the small sample size. (Reviewer-John C. Sabatino, MD).

Keywords: Bile Duct Hamartoma, Biliary Hamartoma, Hepatic Cyst, MRI Findings

Print Tag: Refer to original journal article

Update on EGFR-Based Treatment for Lung Cancer

Gefitinib or Chemotherapy for Non-Small-Cell Lung Cancer With Mutated EGFR.

Maemondo M, Inoue A, et al:

N Engl J Med 2010; 362 (June 24): 2380-2388

First-line treatment of non-small-cell lung cancer with gefitinib was associated with better progression-free survival compared to standard chemotherapy in patients with EGFR-mutated tumors.

Background: Patients with advanced non-small-cell lung carcinoma (NSCLC) who receive standard cytotoxic chemotherapy have an associated response rate of 20% to 35% and a 10- to 12-month median survival time. Data from prior phase 2 trials with gefitinib, a tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR), has shown that patients exhibiting the greatest gefitinib response consistently had significantly higher rates of EGFR mutations. **Objectives**: To compare gefitinib versus standard chemotherapy in previously untreated patients with advanced NSCLC harboring EGFR-sensitive mutations.

Design: Multicenter, randomized phase 3 study. **Participants**: 230 patients diagnosed with metastatic NSCLC with EGFR mutations.

Methods: Participants had not been previously treated with chemotherapy, and were randomly assigned to 1 of 2 treatment arms: gefitinib therapy versus carboplatin-paclitaxel-based chemotherapy. EGFR mutation status was determined using a previously validated polymerase-chain-reaction (PCR)-based detection method. Progression-free survival was considered the primary end point of the study, while overall survival, response rate, and toxic effects were considered secondary end points. The study was prematurely concluded after the first 200 patients had been enrolled, since the planned interim data analysis showed such a significant treatment effect. **Results**: Patients in the gefitinib arm had a significantly higher median progression-free survival compared to patients in the chemotherapy arm (10.8 months vs 5.4 months, respectively; P <0.001). In addition, the gefitinib group showed a significantly higher response rate (73.7% versus 30.7%, P <0.001). For the most part, toxicity profiles of both treatments were predictable: diarrhea and rash were seen more often in the gefitinib group whereas hematologic and neurologic side effects were more commonly seen in the chemotherapy group. However, 3 severe cases of interstitial lung disease were detected in the gefitinib group, and 1 affected patient died as a result (this patient was also a non-smoker). **Conclusions**: First-line therapy with gefitinib for advanced NSCLC harboring EGFR mutations is better in terms of progression-free survival compared to carboplatin-paclitaxel based chemotherapy.

Reviewer's Comments: For the practicing pathologist, there are at least 3 practical points worth underscoring. First, and most obvious, is that gefitinib does seem more effective over conventional chemotherapy in treating advanced NSCLCs that harbor EGFR mutations. Second, pre-treatment mutation analysis of tumor tissue is becoming more crucial to treatment planning. Third, careful validation and consensus adoption of nucleic acid testing methodology should be included in the design and subsequent recommendations of any such study. (Reviewer-T. David Bourne, MD).

Keywords: Non-Small-Cell Lung Cancer, Epidermal Growth Factor Receptor, Gefitinib

Print Tag: Refer to original journal article

Possible New Plasma Biomarker for Acute Myocardial Infarction

Plasma MicroRNA 499 as a Biomarker of Acute Myocardial Infarction.

Adachi T, Nakanishi M, et al:

Clin Chem 2010; 56 (July): 1183-1185

The cardiac-specific microRNA, miR-499, is significantly elevated in patients experiencing acute myocardial infarction.

Background: MicroRNAs (miRNAs) are small RNAs that bind to untranslated regions of mRNA, acting to downregulate and fine-tune the expression of protein-coding genes. To date, >500 miRNAs have been identified in humans. Various body fluids, including plasma, contain detectable levels of miRNA molecules. A recent study using rats reported the detection of a myocardial-specific miRNA in the plasma of animals with acute myocardial injury. **Objectives**: To determine if there are any myocardial-specific miRNAs in humans and to determine whether plasma concentrations of such miRNAs might be useful biomarkers of myocardial injury.

Participants: 10 healthy asymptomatic outpatients and from 29 inpatients. **Methods**: Blood samples were collected participants who had the following types of cardiovascular disease: acute myocardial infarction (AMI; 9 patients), unstable angina pectoris (UAP; 5 patients), and congestive heart failure (CHF; 15 patients). Patients with acute coronary syndromes (AMI and UAP) had all undergone coronary angiography and interventional percutaneous coronary procedures. For AMI patients, blood samples were collected at 2 time points: once within 48 hours of the onset of chest pain, and again just before hospital discharge. The causes of CHF in patients studied included old myocardial infarction (8), dilated cardiomyopathy (4), and valvular heart disease (3). Plasma RNA was isolated and purified from each sample. In order to find myocardial-specific miRNAs, a TaqMan® miRNA array was performed using various human tissues and cultured cells. miRNA plasma concentrations were measured using real-time polymerase chain reaction. **Results**: The TaqMan® miRNA array analysis identified miR-499 as being almost exclusively produced in heart tissue. The limit of detection of miR-499 was 240 copies/100 uL. In control and CHF groups, the miR-499 levels were below limits of detection. In cases of AMI, however, plasma miR-499 concentrations were measurably increased during the acute phase (within 48 hours of chest pain) and undetectable when measured just before hospital discharge. miR-499 levels were also undetectable in patients with UAP. **Conclusions**: Cardiac-specific miRNA, miR-499, may be a useful biomarker of acute myocardial infarction in humans.

Reviewer's Comments: The study is reportedly the first to describe how plasma concentrations of miR-499 may be useful in detecting acute myocardial infarction. Comparison studies of the relative sensitivity and specificity of miR-499 levels with those of cardiac troponin, as well as other established markers of cardiac injury, will be required before concluding that miR-499 detection has clinical utility. (Reviewer-T. David Bourne, MD).

Keywords: Plasma MicroRNA, Acute Myocardial Infarction

Print Tag: Refer to original journal article

Are Signet-Ring Cells in Cytology Always Malignant?

Significance of "Signet-Ring Cells" Seen in Exfoliative and Aspiration Cytopathology.

Wu JM, Ali SZ:

Diagn Cytopathol 2010; 38 (June): 413-418

The presence of signet ring cells in cytology frequently indicates malignancy, and is more likely to indicate malignancy in fine needle aspiration specimens.

Background: A signet-ring cell (SRC) is defined as a cell whose cytoplasm is occupied by a large vacuole which displaces and indents the nucleus to the periphery. SRCs are most commonly associated with malignancy, particularly gastric, ovarian, and breast carcinomas, but can be seen in reactive conditions. The presence of SRCs is particularly problematic in cytology specimens, where the absence of architecture makes misdiagnosis likely.

Objective: To evaluate the significance of SRCs when identified in a variety of cytology sample types.

Methods: Archives were searched for cytology cases with a diagnosis containing the phrase "signet-ring" and material available for review. A variety of specimen types were obtained, including fine needle aspirations (FNA), body cavity fluids and washings, urines, and brushing samples. Slides were reviewed for morphologic features and confirmation of diagnosis. SRCs were defined as large cells, with eccentrically placed nuclei and a clear solitary intracytoplasmic vacuole, which dented the nuclear contour. Follow-up clinical data was obtained from patient charts.

Results: 83 cytology cases with a diagnosis of "signet-ring cell" were identified. The most common sample types were pleural and abdomino-pelvic effusions and washings (56%). FNAs represented 24% of all samples, most commonly from breast and abdominal sites. Cytology interpretation was benign in 16% of cases, malignant in 78%, and indeterminate in 6%. Benign lesions with SRCs were most commonly reactive mesothelial cells. Of malignant lesions, 65% were metastatic adenocarcinoma, most commonly of gastric, esophageal, or breast origin. SRCs seen on FNA were more often indicative of a malignant lesion (90%), followed by paracentesis (73% malignant), and least commonly malignant in washings and urines (50%). In effusions of malignant mesothelioma, SRCs were rare, compared to the abundant SRCs found in effusions involved with metastatic carcinoma.

Conclusions: The presence of SRCs frequently indicates malignancy, and is more likely to indicate malignancy in FNA specimens. However, benign mimics occur, most commonly due to reactive mesothelial cells in effusion cytology.

Reviewer's Comments: The difficulty of SRCs in cytology cannot be understated. In my experience, signet-ring like cells are more frequently seen in benign effusions than found in this study, likely because their presence is recognized as a mimic and not included in the final report. When in doubt, the appropriate application of immunohistochemical stains to verify mesothelial origin should be performed. (Reviewer-Deborah J. Chute, MD).

Keywords: Signet-Ring, Cytopathology, Adenocarcinoma, Mesothelial

Print Tag: Refer to original journal article

Knowing Origin of Multifocal Hepatocellular Carcinomas May Help Treat

Clonal Origin of Multifocal Hepatocellular Carcinoma.

Hodges KB, Cummings OW, et al:

Cancer 2010; May 28 (): epub ahead of print

Multifocal hepatocellular carcinoma appears to be a heterogeneous disease, with some tumors having a monoclonal origin.

Background: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy. The treatment of HCC is limited to interventional radiology techniques and surgical resection, as there is no effective chemotherapy available. Therefore, it is pertinent that a significant number of patients present with multifocal disease. The origin of multifocal HCC is hotly debated, with some authors supporting intrahepatic metastasis, while other authors advocate multiple clonally independent tumors arising due to a field effect.

Objective: To evaluate the clonal relationships of multifocal HCC in a series of patients.

Participants: 31 tumor foci from 12 patients with multifocal HCC.

Methods: HCC was defined as ≥2 tumor foci separated by ≥2 cm of non-neoplastic tissue. Each tumor focus and a sample of normal tissue was microdissected for genomic DNA isolation. Analyzed were 4 highly polymorphic microsatellite loci by polymerase chain reaction (PCR) for loss of heterozygosity (LOH), and was considered present when a >75% loss of intensity of an allele band was present when compared to the normal control. X-chromosome inactivation analysis was performed in 11 female patients, and a non-random inactivation pattern was defined as >75% loss of intensity of an allele band when compared to the normal control. Finally, 3 exons of the *TP53* gene were sequenced, and identical mutations supported common clonal origin.

Results: Of patients tested, 11 were informative regarding clonality by ≥1 test. Of patients tumors, 2 showed discordant LOH patterns and 1 showed discordant X-chromosome inactivation. *TP53* gene mutations were identified in 8 patients, of which 7 showed different point mutations among separate tumor foci, although in 3 patients there were multiple mutations suggesting genomic instability. Overall, 5 patients showed evidence of monoclonal origin (2 presumed to have multiple *TP53* mutations due to genomic instability), while 6 showed evidence to support an independent clonal origin.

Conclusions: The origin of multifocal HCC appears to be heterogeneous, with a significant number of patients having evidence to support monoclonal origin. However, clearly some patients with multifocal HCC actually have independent tumors. Evaluating the origin of multifocal HCCs may become important for treatment decisions in the future, if there is a significant difference in patient prognosis between these patient groups.

Reviewer's Comments: This fascinating study attempts to answer an age old question. However, I place more weight on LOH studies than on discrepant *TP53* mutations. It's a common occurrence in metastatic tumors to pick up additional mutations, especially for *TP53*. Therefore, it is possible that the majority of these patients were of clonal origin (intrahepatic metastasis). (Reviewer-Deborah J. Chute, MD).

Keywords: Liver, Multifocal, Clonality, Loss of Heterozygosity, X Chromosome Inactivation,*TP53*Mutation

Print Tag: Refer to original journal article

Monitoring Residual Myeloma Can Be Complex

Monitoring Residual Myeloma: High-Resolution Serum/Urine Electrophoresis or Marrow Biopsy With Immunohistochemical Analysis?

Tatsas AD, Jagasia MH, et al:

Am J Clin Pathol 2010; 134 (July): 139-144

Serum and urine protein electrophoresis can be used to screen for residual multiple myeloma, possibly deferring bone marrow examination with immunohistochemical analysis.

Background: Monitoring residual multiple myeloma (MM) is a complex process involving invasive and non-invasive techniques. These include serum and urine protein electrophoresis (SPE and UPE), immunofixation (IFX), and free light chain ratios (FLC). Invasive studies include bone marrow (BM)examination via flow cytometry, morphology with immunohistochemical analysis (IHC), cytogenetics and molecular diagnostics.

Objective: To compare the detection of residual MM among various methods.

Participants: 83 consecutive BM biopsies were identified for the evaluation of residual MM.

Methods: Each patient had bone marrow examination with κ and λ light chain immunohistochemistry. Each patient also had SPE and UPE performed at diagnosis and follow-up. An "involved" bone marrow was one with light chain-restricted plasma cells that constituted ≥5% of the total cells by IHC; a light chain restriction was defined as a κ to λ ratio of >5 for κ and <0.5 for λ.

Results: Mean patient age at the time of evaluation for residual MM was 53 years with 47 men and 36 women. Average time from diagnosis of MM to the study was 561 days, although the range was quite broad. There was 100% agreement on the bone marrow biopsy diagnosis (involved or uninvolved) and there was significant correlation between the percentage of monotypic plasma cells in the BM and a serum monoclonal spike. SPE or UPE was positive in all patients with involved BM (sensitivity of 100%) and there was also a negative predictive value of 100%. Of cases, 34 were negative by BM examination but 17 of these were positive by SPE or UPE.

Conclusions: There are a variety of studies for monitoring residual disease in MM and all have their advantages and disadvantages. A recent study showed that BM examination with IHC was the most sensitive method, but this is an invasive and expensive technique. Although there is much interest in FLC ratios, as they may become abnormal before a detectable band appears on SPE or UPE, it is a less specific technique. This study suggests that the primary screening method for patients with minimal residual MM should be SPE and UPE with IFX, with consideration of FLC ratios for negative studies. BM examination should then be performed when there remains high clinical suspicion or for patients with nonsecretory MM. Finally, the authors performed a cost analysis and concluded that the combined cost of BM analysis is 50 times more than the cost of SPE and UPE.

Reviewer's Comments: The challenges to pathologists evaluating bone marrows for residual MM are well appreciated, but we are probably more familiar with these techniques. This study evaluates what I think is a better approach and reserves the challenging bone marrow examination for more necessary cases. (Reviewer-William A. Kanner, MD).

Keywords: Multiple Myeloma, Bone Marrow, Detection

Print Tag: Refer to original journal article

JAK2 Exon 12 Mutations in Polycythemia Vera

Bone Marrow Morphologic Features in Polycythemia Vera With JAK2 Exon 12 Mutations.

Lakey MA, Pardanani A, et al:

Am J Clin Pathol 2010; 133 (June): 942-948

Bone marrow findings in polycythemia vera with *JAK2* exon 12 mutations may differ from those of classical polycythemia vera.

Background: Polycythemia vera (PV) is an erythroid-predominant proliferation resulting from erythropoietin-independent mechanisms. The genetic hallmark of PV is the JAK2V617F gain-of-function mutation. In patients suspected of PV, a positive JAK2V617F test result together with a decreased serum erythropoietin result essentially eliminates the need for other extensive testing to rule out secondary causes. Bone marrow samples from PV patients often show pan-myelosis with erythroid and megakaryocytic hyperplasia. Particularly among the megakaryocytic lineage is the finding of prominent clustering of large megakaryocytes with complex nuclear features. Recently, it has been shown that the majority of mutations in non JAK2V617F cases of PV can be attributed to exon 12 mutations in the *JAK2* gene.

Objective: To describe the bone marrow morphologic features in polycythemia vera with *JAK2* exon 12 mutations.

Participants: 7 consecutive patients identified with PV and *JAK2* exon 12 mutations.

Methods: Clinical and laboratory features were reviewed. Peripheral blood smears, bone marrow aspirates, and biopsy specimens were reviewed. Special stains and immunohistochemistry were also performed. *JAK2* analysis was performed from cells either from the peripheral blood mononuclear cells, granulocytes, or bone marrow cells.

Results: Mean age at diagnosis was 46 years (male to female ratio 4:3). Signs and symptoms included pruritus and/or microvascular complications. Mean hemoglobin concentration was 19.5 g/dL, and all patients had low erythropoietin levels. Conventional karyotypes were normal in all patients. All exon 12 mutations in the *JAK2* gene were identified as heterozygous mutations and no JAK2V617F mutations were present in any case. Bone marrow aspirates and biopsy specimens were hypercellular with a median of 90%. Although erythroid hyperplasia was present, there was no dyserythropoiesis seen. No increase in blasts was identified. All cases had increased numbers of megakaryocytes with atypical features but no cases had prominent tight clusters as expected in typical PV. Reticulin fibrosis was normal to slightly increased in the majority of cases. Immunohistochemical analysis only confirmed the megakaryocytes and no increase in blasts. Median follow-up was 51 months and 1 case evolved into postpolycythemic myelofibrosis and associated osteosclerosis.

Conclusions: Patients with *JAK2* exon 12 positive PV have clinical features similar to classical PV. However, even with the low case numbers in this series, bone marrow features appear different. There are subtle and small megakaryocyte clusters with atypical features (abnormal lobation and chromatin distribution), but certainly not to the extent seen with classical PV.

Reviewer's Comments: Although the vast majority of PV cases are positive for the JAK2V617F mutation, there are well defined cases with only the exon 12 mutation. These cases should be suspected when clinical and laboratory findings are suspicious for PV, the JAK2V617F mutation is negative, and the bone marrow findings demonstrate erythroid hyperplasia and subtle megakaryocytic atypia and/or clustering. (Reviewer-William A. Kanner, MD).

Keywords: Polycythemia Vera,*JAK2*, Bone marrow

Print Tag: Refer to original journal article

Endocervical, Endometrial Adenocarcinoma -- Knowing the Difference

A Panel of 3 Markers Including p16, ProExC, or HPV ISH Is Optimal for Distinguishing Between Primary Endometrial and Endocervical Adenocarcinomas.

Kong CS, Beck AH, Longacre TA:

Am J Surg Pathol 2010; 34 (July): 915-926

A panel of 3 markers can distinguish most endocervical and endometrial adenocarcinomas.

Background: Endocervical and endometrial adenocarcinomas have significant histologic overlap. When precursor lesions are not seen or when tumors involve both portions of the uterus, distinguishing lesions can be especially difficult. Therapy, however, hinges on the correct diagnosis. A number of studies have been published that discuss the use of immunohistochemistry for distinguishing these tumors. Markers include estrogen receptor (ER), progesterone receptor (PR), vimentin, monoclonal carcinoembryonic antigen (mCEA), p16, and in situ hybridization for HPV.

Objective: To investigate the use of these markers in addition to ProExC, which targets cell cycle proteins MCM2 and TOP2A for the distinction of endocervical and endometrial adenocarcinomas.

Methods: A single institution's database was searched for all cases of endocervical adenocarcinoma, endocervical adenocarcinoma in situ, and endometrial adenocarcinoma. A tissue microarray was constructed that contained 214 endometrial adenocarcinomas, 33 endocervical adenocarcinomas, and 36 problematic cases. Whole sections were also used for immunohistochemistry for 38 cases. Immunohistochemistry was performed with antibodies to ER, PR, mCEA, vimentin, p16, and ProExC. HPV in situ hybridization was also performed.

Results: Of cases included in the microarray, 230 were considered to have classic histology and were used as a teaching set. By univariate analysis, p16, ProExC, ER, PR, vimentin and HPV all performed well at distinguishing the 2 tumors. A multivariate model was constructed using all 6 markers that rightly classified 98% of endometrial and 75% of endocervical adenocarcinomas. With test cases, the model rightly classified 97% of endometrial and 56% of endocervical adenocarcinomas. The strongest predictors of site were vimentin, p16 and HPV. HPV and ProExC performed best as single markers. The authors found that 3 marker panels performed the best and that adding more markers did not statistically improve the accuracy of the panels. p16 and vimentin staining worked significantly better with whole sections compared to TMAs. Problem cases for which diagnosis was based predominately on the site of the bulk of tumor showed mixed results suggesting that some tumors were classified incorrectly based on location of tumor bulk. Uterine serous carcinoma was frequently immunoreactive with antibodies to p16 and ProExC and minimal deviation endocervical adenocarcinoma was immunoreactive with antibodies to p16 in 50% of cases and was immunoreactive with antibodies to ProExC in only 25% of cases.

Conclusions: A 3-marker panel may be best for distinguishing endocervical and endometrial adenocarcinomas.

Reviewer's Comments: The authors use statistics to develop panels for distinguishing endometrial and endocervical adenocarcinomas. HPV in situ hybridization has proved to be a helpful test for surgical pathology laboratories. (Reviewer-Edward B. Stelow, MD).

Keywords: Adenocarcinoma, Endocervix, Endometrium, p16, HPV, Immunohistochemistry

Print Tag: Refer to original journal article

Barrett’s Esophagus With Low-Grade Dysplasia Bears Closer Look

Low-Grade Dysplasia in Barrett's Esophagus: Overdiagnosed and Underestimated.

Curvers WL, ten Kate FJ, et al:

Am J Gastroenterol 2010; 105 (July): 1523-1530

Cases originally diagnosed as low-grade dysplasia of the esophagus are frequently found to be free of dysplasia on review.

Background: Barrett's esophagus (BE) develops in patients with chronic reflux esophagitis and is histologically defined by the presence of goblet cell metaplasia within the esophagus. Patients with BE are at increased risk for the development of esophageal adenocarcinoma, a cancer with a dismal 5-year prognosis. It is believed that by monitoring premalignant epithelial changes, screening management can be improved to identify early cancers or high-grade dysplasia so that such disease may be treated early and eradicated. Thus patients with findings indeterminate for dysplasia or low-grade dysplasia undergo increased surveillance.

Objective: To review the natural history of low-grade glandular dysplasia of the esophagus after review of material at an academic center.

Methods: A database of patients diagnosed with BE from 16 hospitals in a metropolitan area was constructed. Hospitals were selected for inclusion into a prospective surveillance study based on the number of BE patients seen. Standardized surveillance and biopsy were initiated for patients willing to enroll in the study. All pathology specimens diagnosed with low-grade dysplasia were reviewed at a single academic center by 2 expert pathologists. Follow-up was obtained and abnormal follow-up histology was also reviewed.

Results: 147 (12%) patients were diagnosed with low-grade dysplasia during the study period. Of biopsies, 75% when reviewed were found to have no dysplasia. Between the 2 pathologists reviewing specimens, there was in interobserver κ-value of 0.5 signifying moderate diagnostic agreement. Of 22 patients with a consensus diagnosis of low-grade dysplasia on review, 8 went on to develop high-grade dysplasia or cancer. Of 14 patients with a consensus diagnosis of indeterminate for dysplasia on review, none went on to develop high-grade dysplasia or cancer. Of 110 patients found to have no dysplasia on review, 2 went on to develop high-grade dysplasia or cancer.

Conclusions: Patients diagnosed with low-grade dysplasia should have their pathology reviewed. Patients with confirmed disease should undergo strict follow-up and possibly be considered for more aggressive procedures.

Reviewer's Comments: It has been known for a long time that there is little interobserver agreement for the diagnosis of low-grade glandular dysplasia of the esophagus. This study confirms that when multiple observers agree on a diagnosis of low-grade dysplasia of the esophagus, the prognostic value of the diagnosis is improved. (Reviewer-Edward B. Stelow, MD).

Keywords: Barrett's esophagus, Dysplasia, Agreement, Outcome

Print Tag: Refer to original journal article

EGFR in Glioblastomas Appears Important

New Pattern of EGFR Amplification in Glioblastoma and the Relationship of Gene Copy Number With Gene Expression Profile.

Lopez-Gines C, Gil-Benso R, et al:

Mod Pathol 2010; 23 (June): 856-865

New patterns of epidermal growth factor receptor amplification may represent an early stage of amplification in glioblastomas.

Background: Epidermal growth factor receptor (EGFR) is among the most frequently amplified genes in cancer, and has been rigorously studied because of the potential for targeted therapy. De novo glioblastoma multiforme (GBM) has overexpressed and amplified, but rarely mutated EGFR. In fact, amplification has been demonstrated in 35% to 70% of glioblastoma, and is usually associated with protein overexpression. This may have prognostic significance.

Objective: To characterize patterns of EGFR amplification with correlation to mRNA levels, protein expression, and other clinicopathologic features.

Participants: 40 cases of GBM subjected to immunohistochemical staining for EGFR (clone H11, Dako).

Methods: Dual-color fluorescent in situ hybridization (FISH) was performed on formalin-fixed, paraffin-embedded tissue sections and primary cultured cells with subsets undergoing quantitative polymerase chain reaction (PCR) and single nucleotide polymorphism (SNP) microarrays.

Results: 40 cases of GBM were analyzed by FISH with 70% having amplification of EGFR. Amplification as double minutes (dmin) were observed in 15 cases (42%), and extra copies of EGFR inserted into the p and/or q arm of chromosome 7 in 11 cases (28%). Of cases, 2 had both of these abnormalities and 12 had none detected. Cases were categorized into group (i) with high-level EGFR amplification and dmin, group (ii) with low-level EGFR amplification and insertions, and group (iii) with no EGFR amplification. The unamplified cases had disomy (n=1), trisomy (n=4) or trisomy/polysomy (n=6) with 90% of these having extra chromosome 7 copies. Higher percentage of cases with amplified EGFR by FISH correlated with a higher gene expression (P <0.01), and the copy-number alterations correlated to the level of EGFR protein expression (P =0.002). Survival curves did not differ significantly between groups, but group (i) had the shortest survival.

Conclusions: GBM had frequent alterations in EGFR gene, particularly dmin and insertions of EGFR copies into chromosome 7. Amplification of EGFR correlates with protein expression.

Reviewer's Comments: The authors propose the importance of EGFR amplification in GBM and suggest that insertion of extra copies into chromosome 7 is an early stage of tumor progression. (Reviewer-Mary T. Galgano, MD).

Keywords: Glioblastoma , EGFR amplification, Insertion, Microarrays

Print Tag: Refer to original journal article

Molecular Profiling May Provide More Data Than CGH on Breast Cancer Genes

Molecular Profiling of Invasive Breast Cancer by Multiplex Ligation-Dependent Probe Amplification-Based Copy Number Analysis of Tumor Suppressor and Oncogenes.

Moelans CB, de Weger RA, et al:

Mod Pathol 2010; 23 (July): 1029-1039

Multiplex ligation-dependent probe amplification analysis evaluates 20 breast cancer genes to provide potential information for therapeutic decision making.

Background: Amplification of the *HER2* gene in breast cancer is detected in less than a third of cases, but confers prognostic information as well as therapeutic implications. Other genes have recently been studied that are thought to also contribute to prognosis and patient specific response to therapy, such as *topoisomerase IIα* (*TOP2A*), *estrogen receptor alpha* (*ESR1*), and *MYC*. *Cyclin D1* (*CCND1*) and *E-cadherin* (*CDH1*) are less understood, but genomic hybridization studies have indicated many different genes with potential valuable information. Multiplex ligation-dependent probe amplification (MLPA) is an easier and faster high throughput polymerase chain reaction (PCR) compared to comparative genomic hybridization (CGH). MLPA can determine a relative gene copy number using minute fragments of DNA, such as those usually obtained from paraffin blocks.This has been validated with *HER2* using comparisons to fluorescence in situ hybridization (FISH) and immunohistochemistry.

Objective: To use MLPA to detect amplifications and/or losses of a variety of breast cancer-related genes previously detected by CGH.

Participants: 104 randomly selected invasive breast cancer cases from a collection in The Netherlands.

Methods: Participants were subjected to MLPA to evaluate amplification of 20 genes. Results were compared to immunohistochemical results for *HER2*, estrogen and progesterone receptor (ER, PR), and other histopathologic characteristics.

Results: Amplification were detected as follows: *MYC* in 48% of tumors; *PRDM14* in 34%; *TOP2A* in 32%; *ADAM9* in 32%; *HER2* in 28%; *CCND1* in 26%; *EMSY* in 25%; *IKBKB* in 21%; *AURKA* in 17%; *FGFR1* in 17%; *ESR1* in 16%; *CCNE1* in 12%; and *EGFR* in 9%. Loss occurred as follows: *CDH1* in 20% and *FGFR1* in 10%. Total number of amplified genes correlated directly with grade and mitotic index. Although *MYC* was amplified in nearly 50%, only a minority were high level. Most of those with *HER2* amplification were high level, and those that were low level were normal by CISH (75%).

Conclusions: MLPA is an alternative to CGH that may provide data on a large number of genes that may be amplified or lost in breast cancer. These data could be used for patient-specific prognosis and therapeutic guidelines.

Reviewer's Comments: The authors describe the methodology of MLPA which can provide patient-specific data, but the specific application of this information should be studied further. (Reviewer-Mary T. Galgano, MD).

Keywords: Breast Cancer, Molecular Profiling, Oncogenes, Amplification

Print Tag: Refer to original journal article