Can Immunohistochemistry Be Applied to Molecular Classification of Lung Ca?

Assessment of EGFR Mutation Status in Lung Adenocarcinoma by Immunohistochemistry Using Antibodies Specific to the Two Major Forms of Mutant EGFR.

Brevet M, Arcila M, Ladanyi M:

J Mol Diagn 2010; 12 (March): 169-176

 EGFR mutant-specific antibodies directed toward the exon 21 L858R EGFR mutation and exon 19 E746-750 deletions represent an effective screening test for predicting lung cancer response to EGFR-tyrosine kinase inhibitors chemotherapy.

Background: Practical lung cancer testing algorithms already include EGFR and KRAS mutation assays and are likely to include EML4-ALK and BRAF assays in the near future.

Objective: To evaluate 2 antibodies “…on a large series of lung adenocarcinomas with molecular data available for EGFR mutation status.”

Materials/Methods: 218 paraffin-embedded lung adenocarcinomas were identified at Memorial Sloan-Kettering Cancer Center (MSKCC). These were enriched to contain 33% positivity for EGFR mutation, greater than the 20% prevalence of EGFR mutations in submitted specimens at MSKCC. Paraffin tumor sections were manually immunostained with 2 rabbit monoclonal antibodies specific for (a) exon 21 L858R EGFR mutation and (b) 15bp exon 19 E746-750 deletion (Cell Signaling Technology) using alkaline EDTA antigen retrieval and overnight antibody incubation at 55°C. Cytoplasmic and/or membrane staining was semiquantitated: 0, no to faint staining intensity in <10% of tumor cells; 1+, faint staining in >10% of tumor cells; 2+, moderate staining; and 3+, strong staining.

Results: Among 218 lung adenocarcinomas, 18 contained EGFR exon 21 substitution mutation L858R (detected by PCR-restriction fragment length polymorphism or mass spectrometry) and 55 cases contained exon 19 deletions. Twenty-two (10.1%) were immunopositive using the L858R mutant-specific antibody and 39 (17.9%) were immunopositive using the exon 19 EGFR deletion-specific antibody. Using a threshold of 1+ staining, the L858R-specific antibody was 95.2% sensitive and 99% specific for molecular L858R mutation. Using a threshold of 2+ staining, the L858R antibody was 76% sensitive and 100% specific. Staining with the exon 19 specific antibody was 100% sensitive and 98.8% specific for 15-bp exon 19 deletions. However, this antibody was less accurate for predicting larger and smaller exon 19 deletions, staining only half of these cases. Based on the size distribution for exon 19 deletion in lung adenocarcinomas, the exon 19-specific antibody is predicted to have an overall sensitivity of 85% and a specificity of 99% for predicting all EGFR exon 19 deletions.

Conclusions: EGFR mutant-specific antibodies are likely to play a key role in molecular classification of pulmonary adenocarcinoma, possibly as a primary screening test requiring reflex DNA testing of negative results.

Reviewer’s Comments: Currently, these antibodies are for research use only. They require both technical and clinical validation before implementation as clinical assay reagents. However, it seems quite likely that will have clinical utility as part of a structured reflex-testing algorithm or for stand-alone testing of biopsy fragments that are too limited for DNA assays. (Reviewer-Guy E. Nichols, MD, PhD).

Keywords: Lung Cancer, EGFR, Adenocarcinoma, Tyrosine Kinase Inhibitors

Print Tag: Refer to original journal article
CD56 expression can be used to aid in the diagnosis of ameloblastoma and to help differentiate this tumor from an odontogenic keratocyst. However, negative stain results should not prompt one to exclude an ameloblastoma diagnosis.

**Background:** Odontogenic lesions can be difficult to diagnose, especially with small amounts of tissue. In general, odontogenic tumors are composed of cellular elements that recapitulate those of normal tooth development, including variable amounts of odontogenic epithelium and the so-called odontogenic ectomesenchyme. Odontogenic tumors include ameloblastoma, ameloblastic carcinoma, ameloblastic fibroma, and odontogenic fibroma, as well as many others. Examples of nonneoplastic lesions include odontogenic keratocyst, radicular cyst, and calcifying odontogenic cyst. A number of immunohistochemical markers, which are expressed at certain time points during tooth development, have been studied in an attempt to identify an antibody that might help distinguish among the various odontogenic lesions. Calretinin, for example, has recently been shown to highlight cells of the stellate reticulum of ameloblastoma. The expression of CD56 (neural cell adhesion molecule; NCAM) has also recently been described in a small series of ameloblastoma cases.

**Objective:** To characterize the expression of CD56 in ameloblastoma and other odontogenic lesions.

**Materials/Methods:** 80 odontogenic lesions were selected for study, including 38 ameloblastomas, 19 odontogenic keratocysts, 9 odontomas, 1 odontoameloblastoma, 1 ameloblastic fibroma, 1 ameloblastic carcinoma, 3 radicular cysts, 4 calcifying odontogenic tumors, 2 adenomatoid odontogenic tumors, 1 odontogenic fibroma, and 1 odontogenic gingival epithelial hamartoma. All lesions were stained using an anti-CD56 antibody. Two pathologists independently reviewed each case and scored the expression as extensively positive (>50% cell positivity), focally positive (1% to 50% cell positivity), or negative (0% cell positivity).

**Results:** 97% (37 of 38) of ameloblastomas showed cell membrane CD56 expression in tumor cell nests. Twenty-one of these cases showed focal staining, while 16 showed extensive staining. In areas of cystic change, inflammation, fusion with the overlying surface epithelium, and acanthomatous differentiation, CD56 expression was lost. One of the 19 odontogenic keratocysts showed focal CD56 positivity, and the single case of odontogenic gingival epithelial hamartoma showed extensive CD56 staining. The single examples of odontoameloblastoma, ameloblastic fibroma, and ameloblastic carcinoma all showed focal CD56 staining.

**Conclusions:** CD56 expression can be used to aid in the diagnosis of ameloblastoma and to help differentiate this tumor from an odontogenic keratocyst.

**Reviewer’s Comments:** It can be challenging to distinguish between an ameloblastoma and an odontogenic keratocyst, especially in patients with large cystic jaw lesions. The findings here do suggest that CD56 provides some support for an ameloblastoma diagnosis. Although not studied here, adding calretinin to the workup might also prove helpful, since it has been shown to label cells of the stellate-reticulum in most cases. An important caveat that is discussed relates to the focal nature of the CD56 staining in >50% of the ameloblastomas, which is that a negative CD56 result in a small biopsy specimen should never prompt one to exclude ameloblastoma with certainty. (Reviewer-T. David Bourne, MD).

**Keywords:** CD56, Odontogenic Lesions, Ameloblastoma

**Print Tag:** Refer to original journal article
Background: Traditionally, the treatment for all renal cortical lesions has been resection, so fine-needle aspiration (FNA) was not considered helpful. However, renal FNA has become increasingly popular, particularly in patients who are candidates for neoadjuvant therapy. Renal FNA has a high sensitivity and specificity in the detection of renal cell carcinoma (RCC), but little information is available regarding the ability of renal FNA to appropriately subtype renal neoplasms.

Objective: To evaluate the ability of renal FNA to appropriately diagnose renal tumors and subclassify RCCs.

Materials/Methods: 143 consecutive renal cortical lesions from adults who underwent partial or radical nephrectomy were included. After surgical resection, ex vivo FNA was performed on the renal mass using a 21-gauge needle. At least 2 passes were performed, and air dried smears were stained with Diff-Quick®. The needle rinse was used to prepare a Papanicolaou-stained Thinprep slide. Each case was evaluated independently by 2 cytopathologists blinded to the surgical diagnosis. The cytologic interpretation was based on previously reported cytologic criteria for renal neoplasms. The surgical diagnosis was considered the gold standard and lesions were classified according to the 2004 World Health Organization classification.

Results: There were 82 cases of conventional clear cell RCC (ccRCC), 17 cases of papillary RCC (PRCC), 13 cases of CRCC, 12 cases of oncocytoma, 3 cases of angiomyolipoma, 3 cases of urothelial carcinoma, and 11 other assorted lesions. A total of 109 cases (77%) were correctly subtyped on the basis of cytology alone. In particular, ccRCC was nearly always correctly interpreted (93% accurate). However, identification of PRCC was much less accurate, with only 47% interpreted correctly, and the other cases interpreted as ccRCC or oncocytoma. While all oncocytomas were correctly interpreted on cytology, only 54% of CRCCs were identified correctly; they remainder were incorrectly interpreted as oncocytoma. Two of 3 angiomyolipomas were correctly identified, but 1 case was incorrectly called ccRCC. Correct classification of other lesions (metastasis, small round blue cell lesion, etc) was poor at 27%, but all were correctly identified as malignant or benign on FNA.

Conclusions: FNA has a high accuracy rate for identifying ccRCC, but a lower accuracy for PRCC and CRCC. This suggests that ancillary studies, such as immunohistochemistry, may have an important role in patients who need accurate subclassification prior to neoadjuvant therapy.

Reviewer's Comments: The difficulty in appropriately classifying renal cortical neoplasms on FNA appears to be due to both the morphologic variability in some tumors (eg, type 1 vs type 2 PRCC) and a lack of sufficient distinguishing features (oncocytomas vs CRCC). In a real clinical situation, it also would be considerably more difficult due to limited cellularity, obscuring blood, and other factors. However, we are still good at correctly identifying malignant from benign processes. (Reviewer-Deborah J. Chute, MD).

Keywords: Renal Fine-Needle Aspiration, Renal Cortical Lesions, Carcinoma

Print Tag: Refer to original journal article
Discuss Transfusion Practices With Your Cardiac Surgeons

Transfusion Requirements After Cardiac Surgery: The TRACS Randomized Controlled Trial.

Hajjar LA, Vincent J-L, et al:

JAMA 2010; 304 (October 13): 1559-1567

There is growing evidence that a more restrictive strategy of blood transfusion is as safe as a liberal transfusion strategy in cardiac surgery patients.

Background: Anemia may be a risk factor for morbidity and mortality following cardiac surgery, thus the rationale for a high rate of blood transfusion during such surgeries. However, transfusion has its own complications and risks, and studies have shown associations between transfusion and high rates of morbidity and mortality in patients after cardiac surgery. Thus, there is the constant investigation into what is the optimal practice regarding blood transfusion in cardiac surgery patients. In the past, the goals have been to maintain hemoglobin of 10 g/dL and a hematocrit of 30 g/dL. However, there have been studies favoring a more restricted use of blood transfusion.

Objective: To publish data from a prospective, randomized, controlled clinical trial to evaluate whether a restrictive blood transfusion was as safe as the more common liberal strategy in patients undergoing elective cardiac surgery. This is the Transfusion Requirements After Cardiac Surgery (TRACS) trial.

Methods: Patients in the restricted transfusion arm received blood products for a hematocrit <24%, and patients in the liberal transfusion arm received blood for a hematocrit <30%. Outcome measures included 30-day all-cause mortality and severe morbidity. The primary morbidities included cardiogenic shock, acute respiratory distress syndrome, and acute renal injury requiring dialysis. Statistical analysis was then performed.

Results: 502 patients were enrolled and evaluated in the study. The mean hemoglobin for both arms was around 13 g/dL. More patients were transfused, as expected, in the liberal arm of the study, with most transfusions coming during surgery and within the first 3 days after surgery. In total, 63% of patients in the study received a red blood cell (RBC) transfusion. For the primary outcome measures, all-cause 30-day mortality and specific morbidities mentioned above occurred in 11% of patients in the restricted-strategy group and 10% of patients in the liberal-strategy group. Regardless of the treatment strategy, the authors found that the number of transfused RBC units was an independent risk factor for a worse outcome. There was a 1.2-fold increased risk of death at 30-days for each transfusion.

Conclusions: In this cardiac surgery patient population, there was no difference in 30-day all-cause mortality and morbidity between patients who were transfused using a liberal versus a restricted strategy. In fact, the findings suggest that one should avoid transfusing solely to correct low hemoglobin.

Reviewer's Comments: There will also be more studies of this kind to assess whether it will be appropriate to be more restrictive in blood transfusion, not just in these patients, but in other scenarios as well. For me, the big picture is that there is a lot more going on with a RBC transfusion than just transfusing red cells for increased oxygen capacity, and there are obvious risks with that. (Reviewer-William A. Kanner, MD).

Keywords: Cardiac Surgery, Blood Transfusion

Print Tag: Refer to original journal article
Intestinal Metaplasia Within Squamo-Oxyntic Gap Is Risk Indicator


Chandrasoma P, Wijetunge S, et al:

Am J Surg Pathol 2010; 34 (November): 1574-1581

A histologic definition of gastroesophageal reflux disease may include the presence of any metaplastic epithelium between the squamous esophagus and gastric oxyntic-type epithelium.

**Background/Objective:** Gastroesophageal reflux disorder (GERD) is common and is defined as the condition that develops when there is a reflux of stomach contents that causes troublesome symptoms or complications. While this definition is somewhat intuitive, it is also independent of any histologic or endoscopic findings. Associated with reflux is columnar metaplasia and dysplasia of the esophageal squamous epithelium and the eventual progression to adenocarcinoma. Frequently (up to 40% of the time), patients with GERD do not develop symptoms until they present with adenocarcinoma. Here, the authors argue for a definition of GERD that includes the presence of the gastric cardia.

**Methods:** All patients seen in a specialized clinic over a 5-year period who had endoscopy with systematic gastric and esophageal biopsies were reviewed by a single pathologist. Epithelial type was described as stratified squamous, cardiac with intestinal metaplasia, cardiac (mucinous cells only), oxyntocardiac (mixture of parietal and mucinous cells below the foveolar region), and gastric oxyntic (no mucinous cells below the foveolar region). The gap between squamous and gastric oxyntic-type mucosa was measured.

**Results:** 1655 patients met the selection criteria. The length of the squamo-oxyntic gap varied from <1 cm to >10 cm. The presence of intestinal metaplasia was correlated with the overall length of the gap and was present in nearly 90% of patients who had a gap >1 cm. With patients who had intestinal metaplasia throughout the entirety of the squamo-oxyntic gaps, goblet cells were more numerous in the more proximal biopsies. A slight majority of patients had both cardiac and oxyntocardiac-type mucosa within the gap, and nearly one-third also had intestinal metaplasia. Cardiac-type mucosa was more common in patients who had longer squamo-oxyntic gaps. The degree of chronic inflammation varied but was most significant in the cardiac-type mucosa. Five percent of patients had *Helicobacter pylori* infection identified in their gastric biopsies.

**Conclusions:** The authors argue that a squamo-oxyntic gap is equivalent to GERD. Its length correlates with the severity of the patients GERD and its distal margin represents the true extent of the esophagus.

**Reviewer's Comments:** The meaning of mucosal changes distal to the tubular esophagus continues to be contentious. These authors now need to correlate their findings with the eventual development of adenocarcinoma. (Reviewer-Eric B. Stelow, MD).

Keywords: Gastroesophageal Reflux Disorder, Metaplasia, Biopsy

Print Tag: Refer to original journal article
For unclear reasons, the follicle-stimulating hormone receptor appears to be widely expressed by tumor endothelial cells within a wide spectrum of human malignancies.

**Background:** Sertoli cells of the testis and granulosa cells of the ovary are the only human tissues that normally express the follicle-stimulating hormone (FSH) receptor. Previous studies have reported expression of the FSH receptor within prostate cancer tumor cells, but no prior reports have documented the pattern of staining within the tumor vasculature.

**Objective:** To assess the expression of the FSH receptor in tumor endothelial cells among various human malignancies.

**Methods:** Tissue samples, from 1336 patients, were collected immediately after surgery from malignant tumors of the prostate, breast, colon, pancreas, urinary bladder, kidney, lung, liver, stomach, testis, and ovary. Tumor tissue was frozen and routinely processed using formalin fixation and paraffin embedding. Histologic analysis of each tumor was performed by 5 study investigators, who diagnosed each case and assigned tumor grades using either World Health Organization or American Joint Committee on Cancer guidelines. Control tissue consisted of grossly and microscopically normal tissue obtained from the surgery specimens. Four different antibodies, all of which were monospecific for the FSH receptor, were applied to each sample (FSHR18, FSHR323, FSHR190, and FSHR225). In situ hybridization, confocal microscopy, and immunoblotting procedures were also performed. Finally, immunoelectron microscopy was performed on previously created prostate cancer tumor xenografts in nude mice after the mice were injected with anti-FSH antibodies coupled to colloidal gold.

**Results:** The FSH receptor was expressed by endothelial cells in all 1336 tumors, regardless of tumor grade. Expression occurred within vessels at the tumor periphery, in a 10-mm thick zone that included vessels within and just outside the tumor. In normal tissue >10 mm from neoplastic tissue, however, no FSH receptor expression was detected. Tumor lymphatics failed to show any evidence of FSH receptor expression. Immunoelectron microscopic analysis of prostate cancer tissue from mouse xenografts confirmed the presence of the FSH receptor within the endothelial cell luminal surfaces as well as coated vesicles, endosomes, and multivesicular bodies.

**Conclusions:** The FSH receptor is widely expressed by tumor-associated endothelial cells within a wide spectrum of human malignancies.

**Reviewer's Comments:** These findings are interesting, although the reason why the FSH receptor is predominantly expressed in vessels at the tumor periphery and in apparently normal vessels near the tumor periphery is unclear. The authors suggest that vessel recruitment by the adjacent tumor vasculature may induce FSH expression. Regardless of the precise mechanism, the demonstrated recognition of anti-FSH receptor antibodies by FSH receptors suggests some practical clinical applications such as in diagnostic imaging and therapeutic drug targeting. (Reviewer-Stacey E. Mills, MD).

Keywords: FSH Receptor, Endothelial Cells, Tumor Vasculature

Print Tag: Refer to original journal article
Study Validates p16 Immunocytochemistry for LSIL, ASCUS

The Sensitivity and Specificity of p16INK4a Cytology vs HPV Testing for Detecting High-Grade Cervical Disease in the Triage of ASC-US and LSIL Pap Cytology Results.
Denton KJ, Bergeron C, et al:

Am J Clin Pathol 2010; 134 (July): 12-21

p16 immunocytochemistry is equally sensitive to HPV DNA testing with superior specificity for predicting which women with low-grade squamous intraepithelial lesion or atypical squamous cell of undetermined significance harbor a high-grade cervical intraepithelial lesion.

Background: Molecular testing for human papillomavirus (HPV) is well established as the method of choice for stratifying patients with atypical cervical cytology results (atypical squamous cells of undetermined significance (ASC-US)) into "repeat cytology" or "colposcopy" management groups. HPV testing is most sensitive for predicting high-grade cervical intraepithelial neoplasia (CIN), albeit at the cost of suboptimal specificity resulting in many negative colposcopic examinations. A growing body of evidence suggests that p16INK4a immunocytochemistry is equally sensitive with superior specificity.

Objective: To correlate immunostaining of liquid-based cervical cytologic samples for protein p16INK4a with HPV testing (Digene High-Risk Hybrid Capture [HC2]) as predictors of biopsy-proven, high-grade cervical intraepithelial neoplasia.

Materials/Methods: 810 liquid-based cervical cytologic samples (ThinPrep test vials) were retrospectively identified in 5 Swiss and Italian pathology laboratories and selected for having a cytologic diagnosis of ASC-US or low-grade squamous intraepithelial lesion (LSIL), sufficient residual liquid sample, and available paraffin tissue blocks from follow-up cervical biopsies taken within 6 months of sampling. Additional cytologic slides were prepared with a T2000 slide processor (Hologic) and stained for p16 using the CINtec Cytology Kit (REF 9521, mtm laboratories). Two interpretive methods were used, each calling a positive result for at least 1 morphologically abnormal p16 immunoreactive cell. HV testing was performed using Digene High-Risk HPV Hybrid Capture (HC2). Results were compared to histopathologic separation into 2 groups, CIN 2 or CIN 3 versus CIN 1 or negative, as the gold standard.

Results: Specimen age ranged from 1 to 36 months. Among 385 cytologic cases of ASCUS, 81 revealed CIN 2/CIN 3 histology and 304 were CIN 1/ negative. Among 425 cytologic cases of LSIL, 141 revealed CIN 2/CIN 3 histology and 284 were CIN 1/ negative. p16 immunocytochemistry showed similar sensitivity (92.6% in the ASCUS group and 92.2% in the LSIL group) to HPV testing (90.1% in the ASCUS group and 95.7% in the LSIL group) for predicting histologic evidence for high-grade CIN. p16 immunocytochemistry showed superior specificity (63% to 71% in the ASCUS group and 37% to 53% in the LSIL group) to HPV testing (38% in the ASCUS group and 18% in the LSIL group) for predicting histologic evidence for high-grade CIN.

Conclusions: p16 immunocytochemistry may serve as the first-line triage method for determining management of women with cytology-proven LSIL and may improve upon or replace HPV testing in the management of ASCUS.

Reviewer’s Comments: This study is the first with sufficient statistical power to validate p16 immunocytochemistry as a test method for management of women with LSIL or ASCUS. Test specificity may have been biased/increased by prolonged specimen age or technical preparation of immunocytochemical slides by the study sponsor. Cytotechnologist interpretation of p16 immunocytochemistry was more sensitive, but less specific, than pathologist interpretation in predicting histologic evidence for high-grade CIN. (Reviewer-Guy E. Nichols, MD, PhD).

Keywords: p16, HPV, Cervical Cancer, LSIL, ASCUS

Print Tag: Refer to original journal article
Hyaluronic Acid Level Helps Predict Fibrosis in Children With NAFLD

Hyaluronic Acid Predicts Hepatic Fibrosis in Children With Nonalcoholic Fatty Liver Disease.

Nobili V, Alisi A, et al:
Transl Res 2010; 156 (October): 229-234

Serum hyaluronic acid level is a predictor of the degree of hepatic fibrosis in pediatric patients.

**Background:** In children and adolescents, the most common chronic liver disease is nonalcoholic fatty liver disease (NAFLD), which affects up to 80% of obese individuals in this age group. The disease is associated with the development of hepatic fibrosis and may eventually progress to cirrhosis. Recent studies have found that, in adults, serum hyaluronic acid (HA) measurement is a good marker for liver fibrosis.

**Objective:** To determine the diagnostic utility of serum HA measurement as a marker of hepatic fibrosis in children with NAFLD.

**Participants/Methods:** 100 consecutive children and adolescents with biopsy-proven NAFLD were enrolled in this cross-sectional study. Exclusion criteria were the use of steatogenic drugs, total parenteral nutrition, alpha-1-antitrypsin deficiency, Wilson's disease, celiac disease, metabolic liver disease, autoimmune liver disease, viral hepatitis, and excessive alcohol intake (defined as ≥20 g/day). Weight, height, and body mass index were determined for each patient. Laboratory measures included alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl-transferase (GGT), glucose, and insulin. Serum HA was determined using an enzyme-linked binding protein assay. Routinely processed liver biopsy samples, which included Van Gieson staining for fibrosis determination, were reviewed by a single pathologist who was blinded to clinical and laboratory data. NAFLD and nonalcoholic steatohepatitis (NASH) were diagnosed using NASH Clinical Research Network criteria, which include assessment of steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis.

**Results:** Liver fibrosis was observed in 65% of children. HA levels greater ≥2100 ng/mL were associated with a high likelihood of fibrosis (89%; 95% CI, 75% to 100%). There was no association between the stage of fibrosis and the degree of hepatocyte ballooning, inflammation, or steatosis.

**Conclusions:** In a pediatric population with NAFLD, serum HA level is a predictor of the degree of hepatic fibrosis. This information may ultimately be useful in terms of screening children at-risk for progressive liver disease.

**Reviewer's Comments:** Liver biopsy currently represents the gold standard test for the diagnosis of NASH and for the accurate assessment of liver fibrosis. Although it is a relatively straightforward procedure, liver biopsy in children is considered riskier than in the adult population. Thus, a noninvasive marker of fibrosis would be welcome. (Reviewer-T. David Bourne, MD).

Keywords: Nonalcoholic Fatty Liver Disease, Hepatic Fibrosis, Hyaluronic Acid

Print Tag: Refer to original journal article
Are Isolated Tumor Cells Predictive of Worse Outcome in Lung Ca Patients?

The Presence of Isolated Tumor Cells and Micrometastases in the Intrathoracic Lymph Nodes of Patients With Lung Cancer Is Not Associated With Decreased Survival.

Marchevsky AM, Gupta R, et al:

Hum Pathol 2010; 41 (November): 1536-1543

There is little evidence to support the routine performance of immunohistochemistry when evaluating mediastinal and hilar lymph nodes for metastatic non-small cell lung cancer.

**Background:** Cancer stage is one of the most important predictors of patient outcome for non-small cell lung carcinoma (NSCLC). A controversial area in tumor staging is the role of micrometastases and isolated tumor cells in hilar and mediastinal lymph nodes. This topic has been extensively explored for other neoplasms, such as breast cancer, but little is known regarding the prognostic impact and importance of occult lymph node metastasis in patients with NSCLC.

**Objective:** To evaluate one institution's experience with isolated tumor cells and micrometastases in clinical stage I NSCLC patients, as well a review of the literature using meta-analysis.

**Participants/Methods:** 266 patients with clinical stage I NSCLC who underwent lobectomy and nodal dissection were included. Hilar and mediastinal lymph nodes from these patients were evaluated for metastatic tumor using H&E stain and immunohistochemical staining for AE1/AE3. According to the American Joint Commission on Cancer guidelines, small nodal metastases were classified according to size as "isolated tumor cells" when <0.2 mm, "micrometastases" when between 0.2 mm and 2.0 mm, and "metastasis" when >2.0 mm. Lymph nodes with isolated tumor cells were classified as pN0(i+), lymph nodes with micrometastases were classified as pN1(mi) or pN2(mi), and lymph nodes with metastases were classified as pN1 or pN2, according to the lymph node location. Finally, a meta-analysis of 13 studies reporting isolated tumor cells or micrometastases in NSCLC patients was performed.

**Results:** After H&E and immunohistochemical examination, the lymph node status was pN0 for 160 patients, pN0(i+) for 7 patients, pN1(mi) for 27 patients, pN1 for 44 patients, and pN2(mi) for 28 patients. All isolated tumor cells and micrometastases were only detected with immunohistochemistry. There was a statistically significant different in overall survival between patients with pN0 and pN1 disease (median, 70 months vs 39 months). However, there was no statistical difference in survival between patients with pN0 and those with pN0(i+), pN1(mi), or pN2(mi) disease, or between patients with pN1 and pN2(mi) disease. On meta-analysis, an additional 835 patients with NSCLC from the literature also showed no significant prognostic differences between patients with pN0 disease and those with pN1(mi) or pN2(mi) disease. A power analysis demonstrated that >2000 patients would be needed to detect survival difference in NSCLC patients with micrometastases.

**Conclusions:** Currently, there is no evidence to support a difference in survival between patients with negative mediastinal lymph nodes and those with isolated tumor cells and/or micrometastases. However, even using meta-analysis, the studies to date are underpowered to detect small differences in patient outcome.

**Reviewer's Comments:** Despite the power problems with this study, this article highlights that there is little evidence to support routinely performing immunohistochemistry when evaluating mediastinal and hilar lymph nodes for metastatic NSCLC. (Reviewer-Deborah J. Chute, MD).

**Keywords:** Micrometastases, Isolated Tumor Cells, Lung Cancer, Prognosis

**Print Tag:** Refer to original journal article
Especially with lesions on the extremities, epithelioid hemangioma may mimic cutaneous lymphoma, especially when there are atypical CD30+ lymphocytes present.

**Background:** Epithelioid hemangioma (EH) is a vascular lesion also known as angiolymphoid hyperplasia with eosinophilia. Typically, this is a lesion of the head and neck area, but it is not rare to find this lesion on the extremities. It is because of the exuberant lymphoid response that EH may be mistaken for a lymphoproliferative disorder, especially when it is present in an atypical location.

**Objective:** To emphasize the importance of considering a diagnosis of epithelioid hemangioma when faced with a lesion on the extremities with an inflammatory component.

**Methods:** The authors retrospectively reviewed cutaneous biopsies from 4 patients. The clinical features were also reviewed. The histologic features were reviewed in detail, and all cases were stained with CD30 and CD34 (3 of 4 cases were also stained with CD31 and CD163).

**Results:** In this small study, the age range of the 3 men and 1 woman was from 33 to 78 years. All lesions were from the upper extremity. The clinical impressions were of PLEVA versus lymphomatoid papulosis, persistent tick bite, persistent bug bite, and no impression, which was given for the fourth case. Follow-up data showed no evidence of disease in all cases (3 to 38 months). On histologic examination, the vascular component demonstrated a lobular pattern of small capillary-sized vessels centered around a "feeder" vessel. The endothelial cells lining the larger vessels were notable for an epithelioid appearance, and in some areas, formed a solid component (ie, a histiocytoid appearance). The inflammatory component was a mixed infiltrate in a perivascular and periadnexal distribution. It was composed of lymphocytes, histiocytes, eosinophils, mast cells, and plasma cells and was usually more exuberant at the periphery or deep aspects of the lesion. In all cases, there were large transformed lymphocytes that were CD30+, a finding that usually raises suspicion for a lymphoproliferative disorder, especially in dermatopathology.

**Conclusions:** Cases such as those presented in this paper, clinically resemble the scenario that may occur with a primary cutaneous CD30+ T-cell lymphoproliferative disorder, a cutaneous anaplastic large cell lymphoma, or lymphomatoid papulosis. The authors emphasize that the key to diagnosis is recognition of the vascular component of EH. This is also true for other diagnoses, such as the resulting pathology due to an insect/arthropod bite. Thus, the finding of CD30+ cells in lymphocytic infiltrates needs to be correlated closely with the histologic findings and clinical presentation.

**Reviewer’s Comments:** It may be very easy to not consider vascular lesions in one’s differential diagnosis. This article discusses a very distinct diagnosis and gives some depth about potential pitfalls within the differential diagnosis. What is most striking and I think most important is that there can be atypical CD30+ lymphocytes in an epithelioid hemangioma. (Reviewer-William A. Kanner, MD).

**Keywords:** Epithelioid Hemangioma, CD30, Angiolymphoid Hyperplasia, Eosinophilia

Print Tag: Refer to original journal article
Chronic duodenitis in patients with ulcerative colitis who have undergone colectomy is associated with pouchitis.

**Background:** Ulcerative colitis is usually thought of as a disease that affects only the mucosa of the rectum and colon. Recently, some have shown that chronic inflammatory lesions of the upper gastrointestinal tract are present with increased frequency in patients with ulcerative colitis compared to control patients.

**Objective:** To ascertain and describe the types of inflammatory conditions seen in the stomachs, esophagi, and duodenums of patients with ulcerative colitis.

**Participants/Methods:** 69 patients who had biopsy-confirmed ulcerative colitis and had biopsies of the esophagus, stomach, and/or duodenum and were seen at a single institution were included. Biopsies were compared to those of a control group of patients who did not have ulcerative colitis. All biopsies were taken randomly rather than by protocol. The biopsies were reviewed by 3 pathologists, and all histologic features were recorded.

**Results:** 16 of the 69 patients were <18 years old. Twenty-four patients had biopsies of the esophagus, 66 of the stomach, and 40 of the duodenum. Ten of the ulcerative colitis patients had undergone complete colectomy. With esophageal biopsies, control patients were more likely to have ulcers; no other changes were significant. With gastric biopsies, ulcerative colitis patients were more likely to have a focal gastritis than were the controls, defined as a localized collection of lymphocytes, neutrophils, and macrophages surrounding at least 1 pit, neck, or gland. This was seen in nearly 30% of the patients with ulcerative colitis. Ulcerative colitis patients were also more likely to have basal mixed inflammation and a superficial plasmacytosis than controls in their gastric biopsies. Control patients were more likely to have *Helicobacter pylori* gastritis. With duodenal biopsies, patients with ulcerative colitis were more likely to have a diffuse chronic duodenitis characterized by diffuse mucosal chronic inflammation, mainly plasmacytic, with a variable number of neutrophils either within the lamina propria or involving the crypts. Architectural distortion, similar to that seen in the colon with chronic ulcerative colitis, was also present. These changes were present in 10% of the biopsies of ulcerative colitis patients. This duodenitis occurred only in patients who had previously undergone colectomy, and all of these patients had also developed a pouchitis.

**Conclusions:** Although most patients who have ulcerative colitis have normal upper gastrointestinal biopsies, some may have a focal gastritis or chronic duodenitis. The chronic duodenitis appears to be closely related to the development of pouchitis.

**Reviewer's Comments:** The development of a duodenitis in patients who have had previous surgery for ulcerative colitis may cause one to wonder if the patient had originally truly had Crohn’s disease. The documentation of this phenomenon by a major academic group of gastrointestinal pathologists will help assure pathologists and clinicians that this need not be the case. (Reviewer-Edward B. Stelow, MD).

**Keywords:** Duodenum, Inflammation, Ulcerative Colitis, Pouchitis

**Print Tag:** Refer to original journal article
Patients with autoimmune metaplastic atrophic gastritis most frequently have hyperplastic polyps or carcinoid tumors when they have mass lesions.

**Background:** Autoimmune metaplastic atrophic gastritis (AMAG) and pernicious anemia are now known to be conditions that can affect individuals of all races, usually those older in age. The diagnosis of AMAG is important not only because it can eventually be associated with pernicious anemia, but because it is a precursor lesion for the development of gastric adenocarcinoma. Histologically, AMAG is characterized by chronic inflammation of the body of the stomach with loss of parietal cells and re-epithelialization by pyloric or intestinal-type epithelium. Patients are then at risk for development of polyps, adenocarcinoma, and carcinoid tumors.

**Objective:** To review the experience of 1 tertiary care center with gastric biopsies and lesions from patients with AMAG.

**Methods:** All non-consultation surgical pathology cases seen at a single institution over a 20-year period diagnosed as autoimmune gastritis or AMAG were reviewed. Criteria included chronic inflammation of body mucosa with oxyntic gland destruction and pyloric or intestinal-type metaplasia and enterochromaffin-like cell hyperplasia. All polyps and other lesions seen in these patients were reviewed.

**Results:** 461 patients with AMAG were seen over the 20-year period. These cases accounted for approximately 1% to 3% of the gastric biopsies seen from all races, and there were approximately twice as many women as men. The median patient age was 67.5 years; however, patients as young as 18 years of age were diagnosed with the disease. The median age for non-white patients was younger than it was for white patients. A total of 240 grossly identifiable lesions were seen in 31% of the patients with AMAG. These included 138 hyperplastic polyps, 20 fundic gland-type polyps, 18 intestinal-type adenomas, 3 pyloric gland adenomas, 46 carcinoid tumors, 1 gastrointestinal stromal tumor, 3 lymphomas, and 11 adenocarcinomas. The majority of adenocarcinomas were poorly differentiated and many had signet ring-cell features. All patients diagnosed with lymphomas were noted not to have *Helicobacter pylori* infection.

**Conclusions:** AMAG occurs over a wide age range and clinical spectrum. The most common mass lesions seen with the disease include hyperplastic polyps and carcinoid tumors. Although pyloric-type adenomas have been described in AMAG patients, they appear to arise very infrequently.

**Reviewer's Comments:** This study confirms much of what is known about AMAG. It stresses again the importance of looking for the disease when either hyperplastic polyps of carcinoid tumors are sampled within the stomach. (Reviewer-Edward B. Stelow, MD).

Keywords: Gastritis, Autoimmune, Atrophic, Polyp, Adenoma

Print Tag: Refer to original journal article
Remember Your PTLD Subtypes

Pathologic and Clinical Features of Hodgkin Lymphoma–Like Posttransplant Lymphoproliferative Disease.

Krishnamurthy S, Hassan A, et al:


Hodgkin lymphoma posttransplant lymphoproliferative disorder (PTLD) is the rarest of PTLD subtypes.

**Background:** Posttransplant lymphoproliferative disorder (PTLD) is an abnormal proliferation of lymphoid cells that occurs in the setting of transplantation as a result of immunosuppression. Most PTLD cases are driven by Epstein-Barr virus (EBV) infection. The current WHO classification system for PTLD recognizes 4 categories: early lesions; polymorphic PTLD; monomorphic PTLD; and Hodgkin lymphoma (HL)-type PTLD. HL type PTLD is rare, and it includes true HL-PTLD and the so-called HL-like PTLD. Unlike HL-PTLD, HL-like PTLD has Reed-Sternberg (RS) cells, which lack CD15 staining and co-express various B-cell antigens.

**Objective:** To compare the histologic pattern, immunophenotypic pattern, EBV status, and clinical behavior of HL-like PTLD cases with cases of monomorphic B-cell PTLD.

**Methods:** Formalin-fixed, paraffin-embedded tissue from 11 cases was reviewed for the study (6 HL-like PTLD and 5 monomorphic B-cell PTLD). Clinical data collected for each case included the type of organ transplant received, the interval between transplant and lymphoma diagnosis, EBV status, disease stage, treatment, and patient outcome. Immunohistochemistry using the following antibodies was performed: CD45 (LCA), CD20, CD79a, CD30, CD15, BOB.1, Oct-2, and Fascin. In situ hybridization for EBER was also performed.

**Results:** Unlike monomorphic B-cell PTLD, cases of HL-like PTLD resembled classical HL in that there were well-visualized RS-like cells and a mixed background of lymphocytes and other scattered inflammatory cells. By IHC, the RS-like cells in HL-like PTLD were CD45-negative. Unlike the RS cells in classical HL, however, the RS-like cells in HL-like PTLD were generally CD15-negative. Furthermore, like the monomorphic B-cell PTLD cases, the RS-like cells of HL-like PTLD were generally strongly positive for the B-cell associated markers BOB.1 and Oct-2. ISH-EBER was positive in all cases from each group. Half of the patients diagnosed with HL-like PTLD relapsed or died following treatment, while all patients diagnosed with monomorphic B-cell PTLD showed no evidence of disease at the time of study completion.

**Conclusions:** Although HL-like PTLD cases have similar histologic features in common with HL-PTLD, the immunophenotypic expression pattern and more aggressive clinical course suggests that HL-like PTLD represents a distinct clinical entity worthy of subclassification.

**Reviewer's Comments:** Key things to remember from this article are that classical Hodgkin lymphoma PTLD is the rarest of PTLD subtypes, and that you really need to be strict with your interpretation of Reed-Sternberg cell immunostaining; RS cells in classical cases really should be CD45-, CD15+, and CD30+. If the RS cells are CD15-, you should strongly consider the possibility of a so-called HL-like PTLD and proceed with additional B-cell associated markers. A more careful stage-for-stage comparison with more cases should be performed before concluding with certainty that HL-like PTLD cases are more clinically aggressive. (Reviewer-T. David Bourne, MD).

**Keywords:** Posttransplant Lymphoproliferative Disorder, Hodgkin Lymphoma

Print Tag: Refer to original journal article
More to the Story of *H. pylori*

*Helicobacter pylori* Accelerates Hepatic Fibrosis by Sensitizing Transforming Growth Factor-β1-Induced Inflammatory Signaling.

Ki M-R, Goo M-J, et al:

Lab Investigation 2010; 90 (October): 1507-1516

In a murine model, *Helicobacter pylori* infection accelerates hepatic fibrosis when coupled with pre-existing CCl₄-induced liver injury via enhanced proinflammatory transforming growth factor-beta1 activity.

**Background:** Since its cultivation in 1982, *Helicobacter pylori* have been recognized as a causal agent in a variety of gastric and duodenal diseases. *H. pylori* infection has been associated with systemic inflammation, and studies have shown that patients with cirrhosis and hepatocellular carcinoma (HCC) have a higher frequency of *H. pylori* infections; genomic sequences of *H. pylori* have even been detected in liver tissue from patients with HCC. In mice, the newly described *Helicobacter* sp., called *H. hepaticus*, has been proven to cause chronic hepatitis and HCC. A recent study supports the role that *H. pylori* might play in the pathogenesis of cirrhosis due to its acceleration of CCl₄-induced hepatic fibrosis.

**Objective:** To test the hypothesis that *H. pylori* accelerates hepatic fibrosis through increased inflammatory signaling.

**Methods:** 8-week-old female mice were assigned to 1 of 4 groups: normal control group; *H. pylori*-infected group; CCl₄-treated group; and *H. pylori* + CCl₄-treated group. *H. pylori* were administered via orogastric inoculation, while 10% CCl₄ was administered via intraperitoneal injection. After 15 weeks in the study, blood specimens were drawn to measure serum levels of tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta 1 (TGF-β1). Formalin-fixed, paraffin-embedded samples of liver tissue were stained using antibodies against proliferating cell nuclear antigen (PCNA). A quantitative analysis of apoptosis was also determined. Immunoblot studies were performed using antibodies against mitogen-activated protein kinases (MAPKs), p53-related proteins, antioxidants, and various proinflammatory cytokines.

**Results:** A number of results were observed: mice in the *H. pylori* + CCl₄ group had significantly elevated TNF-α and TGF-β1 levels, *H. pylori* was shown to increase MAPK signalling and to augment mRNA expression of proinflammatory cytokines, and *H. pylori* was shown to modulate oxidative stress via increased catalase levels.

**Conclusions:** Endotoxins produced by *H. pylori*, which are transported to the liver via the portal vein, may accelerate the development of hepatic fibrosis when coupled with pre-existing CCl₄-induced liver injury by increasing the proinflammatory activity of TGF-β1.

**Reviewer’s Comments:** The development of hepatic fibrosis and, ultimately cirrhosis, is a dynamic process. We are classically taught that the mechanism of fibrosis formation involves the proliferation of hepatic stellate cells and their subsequent activation into cells with myofibroblastic properties. The potential contributing role played by *H. pylori* infection is quite interesting. (Reviewer-T. David Bourne, MD).

**Keywords:** *Helicobacter pylori*, Hepatic Fibrosis, Cirrhosis

**Print Tag:** Refer to original journal article
What Is Association Between Cup-Like Nuclei and AML With Mutation of NPM1 Gene?

Is the Association of "Cup-Like" Nuclei With Mutation of the NPM1 Gene in Acute Myeloid Leukemia Clinically Useful?

Bennett JM, Pryor J, et al:

Am J Clin Pathol 2010; 134 (October): 648-652

There is an association between "cup-like" nuclei and acute myeloid leukemia with mutation in the NPM1 gene, but it should not replace molecular testing.

**Background:** The most common genetic alteration in acute myeloid leukemia (AML) is mutation in the nucleophosmin gene (NPM1). The common characteristics of AML with NPM1 mutations include a normal karyotype, an internal tandem duplication of the FLT3 gene (FLT3-ITD), a higher WBC count in peripheral blood and blast count in the bone marrow, lower expression of CD34, and female sex. NPM1 mutations correlate with improved outcome in the absence of FLT3-ITD mutation. NPM1 mutations have been associated with a distinct feature, termed "cup-like" nuclei. These nuclear invaginations (NIs) have been studied previously and have shown to be associated with less expression of HLA-DR and a normal karyotype.

**Objective:** To evaluate the concordance of the histologic finding of nuclear invaginations (NIs) in patients with AML in predicting the presence of mutations in the NPM1 gene.

**Design/Methods:** In this retrospective study, samples from 81 patients were identified over a 3-year period. NPM1 mutations were detected using a fluorescence-based assay. Two pathologists reviewed slides independently, and subsequently, a pathology trainee reviewed the material to determine if this histologic finding was teachable. The reviewers defined a blast with NI as a spherical object that had compressed an otherwise round nucleus >25% of its diameter.

**Results:** Of the 66 cases that were included in the study, 17 (26%) possessed the NPM1 mutation. In 15 of the 17 cases, a complete remission was achieved, and 6 of these cases have not relapsed to date. The authors found that using a cutoff of 7% NI blasts led to excellent specificity but a sensitivity of only 30%. A receiver operating characteristic curve showed that lowering this cutoff would significantly alter the specificity. Whenever there were >10% NI blasts, an NPM1 mutation was highly likely. However, the mutation was present in more than half the cases in which there were NI blasts ranging from 1% to 6%.

**Conclusions:** The authors show that the histologic finding of "cup-like" nuclei is highly reproducible and not difficult to learn. However, the data demonstrate that the association is not strong enough to be clinically useful (ie, these blasts are not specific to AML with NPM1 mutations. Thus, while the association is present, it does not substitute for the genetic analysis of NPM1.

**Reviewer's Comments:** This article illustrates how molecular diagnostics is really its own entity. Although the histology of some AML blasts may suggest the molecular finding of a mutation in the NPM1 gene, it is not reliable and not a surrogate for the molecular tests. (Reviewer-William A. Kanner, MD).

Keywords: Acute Myeloid Leukemia, NPM1, Nuclear Invagination

Print Tag: Refer to original journal article
Immunohistochemistry for C3d is a valuable tool for the diagnosis of bullous pemphigoid.

**Background:** Autoimmune bullous diseases of the skin require clinicopathologic correlation and, importantly, frozen tissue for direct immunofluorescence (DIF). DIF detects tissue-bound antibodies in skin biopsies. There are characteristic patterns that can be readily identified, but it would be less cumbersome to use immunohistochemistry (IHC) on paraffin-embedded tissue. To this end, the detection of complement proteins has been investigated. C3d forms as a result of the deactivation of C3b and is an indication that there has been complement activation. While other complement components disappear, C3d remains attached to the target cell and can be detected with IHC.

**Objective:** To investigate C3d IHC in autoimmune bullous diseases of the skin.

**Methods:** A variety of cases and controls were selected spanning the years 2006 to 2009. The cases included bullous pemphigoid (BP), pemphigus, dermatitis herpetiformis Duhring, linear Immunoglobulin A (IgA), and mixed BP and linear IgA. All diagnoses were based on histopathologic findings with DIF. IHC was performed and reviewed blindly by 2 pathologists. Staining was scored as 0 to 3+ with scores of 1, 2, and 3 defining a positive result. If C3d positivity was present only within the blister cavity or at the roof/base of the blister with basement membrane deposition in the perilesional skin, it was considered a negative result.

**Results:** In total, 97% of BP cases showed linear deposits of C3d along the basement membrane. Only one case was negative. There was a spread in the positivity with 7, 14, and 10 cases staining 1, 2, and 3+, respectively. All 3 cases of pemphigoid gestationis (PG) showed linear C3d positivity along the basement membrane. Finally, both cases of the mixed form of linear IgA dermatosis and BP showed similar reactivity. Of the other entities, only 3 of 14 cases (21%) of pemphigus demonstrated reactivity, both representing cases of pemphigus vulgaris. All other entities (including the controls) were negative.

**Conclusions:** While there have been only a very few studies using IHC for detecting complement and immunoglobulin deposition in the skin, this study shows that this may be a valuable tool. Unfortunately, there were no cases of epidermolysis bullosa acquisita in this study, which can appear very similar to BP. However, while IHC cannot replace DIF in all autoimmune bullous disorders, it is a useful tool for BP and PG, and a positive result in BP may prompt serologic confirmation.

**Reviewer's Comments:** Outside of a busy dermatopathology service, I am unsure how often pathologists study these blistering disorders. This article is appealing in that it discusses the main bullous skin diseases and helps one better understand the usefulness for markers such as C3d. (Reviewer-William A. Kanner, MD).
Increased EMP2 expression may predict development of endometrial carcinoma in patients with endometrial hyperplasia.

**Background:** Endometrial cancer is the most common gynecologic malignancy. The progression from hyperplasia to endometroid adenocarcinoma has been well studied, but only a fraction of patients with endometrial hyperplasia progress to malignancy. Currently, there is no reliable method to distinguish which patients will progress, so most patients with pre-neoplastic disease undergo conservative management, including hormonal treatment. Epithelial membrane protein 2 (EMP2) is a biomarker that was recently identified as a marker for endometrial carcinoma progression and more aggressive endometrial tumor behavior.

**Objective:** To evaluate the expression of EMP2 in a variety of benign and pre-neoplastic endometrial conditions as a potential marker of future endometroid adenocarcinoma development.

**Methods:** A tissue microarray (TMA) was constructed from endometrial biopsies and curettages from women with a variety of benign endometrial conditions (proliferative, secretory, or polyp), pre-neoplastic conditions (simple or complex hyperplasia with or without atypia), and primary endometrial endometrioid adenocarcinoma. Each sample was represented by 3 tissue cores on the TMA. The majority of patients included in the study had follow-up biopsies (metachronous samples) also included in the TMA, of which a subset had disease progression to endometrioid adenocarcinoma. Immunohistochemical staining for EMP2 was performed on the TMA, and the percentage of cells stained was scored.

**Results:** 207 patients with 535 surgical specimens were included in the TMA. The samples included 231 benign biopsies, 195 pre-neoplastic biopsies, and 109 endometrioid adenocarcinomas; 150 patients had at least 2 specimens, and 46 patients had disease progression to endometrial adenocarcinoma. EMP2 immunohistochemistry showed cytoplasmic and/or membranous staining in 52% of tissue samples. There was a step-wise increase in relative EMP2 expression from benign to pre-neoplastic to endometrioid adenocarcinoma. There was some degree of heterogeneity of EMP2 expression in the pre-neoplastic group; when subgroup analysis was performed, there was a striking increase in EMP2 expression in pre-neoplastic lesions from patients who eventually developed endometrioid adenocarcinoma. However, the EMP2 expression level did not correlate with disease progression in benign biopsies.

**Conclusions:** EMP2 protein expression is potentially an early predictor of endometrial cancer development in patients with pre-neoplastic endometrial lesions (hyperplasia).

**Reviewer's Comments:** This intriguing paper suggests that there may be a way to distinguish which endometrial hyperplasias are most likely to progress to cancer. My major critique of this article is that the amount of tumor cells staining for EMP2 to be considered "positive" within the progression group was unclear, which could make reproducing this study difficult. (Reviewer-Deborah J. Chute, MD).

**Keywords:** Epithelial Membrane Protein-2, Endometrial Cancer, Tissue Microarray

**Print Tag:** Refer to original journal article
Can We Treat Malignant Rhabdoid Tumors With Imatinib?

The Tyrosine Kinase c-Abl Promotes Proliferation and Is Expressed in Atypical Teratoid and Malignant Rhabdoid Tumors.

Koos B, Jeibmann A, et al:
Cancer 2010; 116 (November 1): 5075-5081

Malignant rhabdoid tumors appear to require c-Abl expression for growth, and tyrosine kinase inhibitors such as imatinib may be a viable alternate therapy for patients with this disease.

Background: Malignant rhabdoid tumors can occur in the central nervous system (atypical teratoid/rhabdoid tumors, or AT/RT) or in the kidney and extra-renal tissues (extra-cranial malignant rhabdoid tumors). These tumors have a dismal prognosis, and a limited improvement in survival can be obtained using very aggressive multi-modality therapy that is highly toxic. Alternate, less-toxic therapies are highly desirable, and limited information is available on the role of tyrosine kinases in malignant rhabdoid tumors.

Objective: To investigate the expression and function of tyrosine kinase inhibitors in rhabdoid tumors.

Methods: 18 extra-cranial malignant rhabdoid tumors and 5 AT/RTs with formalin-fixed, paraffin-embedded tissue available were studied. Immunohistochemistry was performed on a tissue microarray of these tumors with antibodies against the platelet-derived growth factor (PDGF)-α receptor, PDGF-β receptor, c-kit protein, and c-Abl protein. Chromogenic in situ hybridization was performed on the tissue microarray to determine if gene amplification (>6 signals per nucleus) was present for PDGF-α receptor, PDGF-β receptor, and c-Abl. Cell cultures from 2 rhabdoid tumor cell lines were used to evaluate the expression of c-Abl protein using immunoblot analysis, and small interfering RNAs specific for c-Abl inhibition were used on the cell lines to evaluate the effect of c-Abl knock-down on tumor proliferation. Finally, the cell lines were incubated with various concentrations of imatinib to evaluate the effect on tumor viability and proliferation.

Results: Immunohistochemical cytoplasmic expression of c-Abl was present in all tumors tested, although it was stronger in the extracranial malignant rhabdoid tumors. Other tyrosine kinase inhibitors showed more variable expression, with only 22% positive for PDGF-α receptor, 22% positive for PDGF-β receptor, and no cases expressing c-kit. There was no evidence of gene amplification for any target in any case. High levels of c-Abl were present on immunoblot from both rhabdoid cell lines. Knock-down of c-Abl mRNA by small interfering RNAs resulted in a significant decrease in mRNA expression and a significant decrease in tumor proliferation. Imatinib showed a significant dose-dependent decrease in proliferation of rhabdoid tumor cell lines at 48 hours.

Conclusions: C-Abl likely plays an important role in the biology of malignant rhabdoid tumors. Tyrosine kinase inhibitors, such as imatinib, may be of utility in the treatment of these aggressive tumors.

Reviewer’s Comments: This well-designed study is a large step forward in the potential treatment of malignant rhabdoid tumors, and it also shows the importance of pathologists in the development of new therapies. The next step will be a pilot clinical trial to determine if imatinib has any activity in vivo in patients with rhabdoid tumors. (Reviewer-Deborah J. Chute, MD).

Keywords: Tyrosine Kinase, c-Abl, Imatinib Mesylate, Pediatric Oncology, Rhabdoid Tumor

Print Tag: Refer to original journal article
Granulocyte analysis is more accurate than monocyte and red cell analysis for measuring GPI-deficient cells. Granulocytes identified by light scatter, and CD15 back-gating can be assessed for abnormal decreases in CD24/FLAER staining.

**Background:** Paroxysmal nocturnal hemoglobinuria (PNH) typically presents with complement-mediated hemolysis and often with life-threatening thrombosis or bone marrow failure. PNH arises from acquired stem cell mutations, leading to deficiency of cell membrane proteins linked to glycophosphatidylinositol (GPI) anchors. PNH is a highly morbid disease that can be treated by the monoclonal antibody eculizumab, making diagnostic testing vital.

**Objective:** To improve technical, interpretive, and reporting standards for paroxysmal nocturnal hemoglobinuria testing.

**Methods:** Consensus guidelines were developed at the 2008 Clinical Cytometry Society Meeting. Recommendations provide novel technical, interpretive, and reporting guidelines to improve the sensitivity, reproducibility, and clinical utility of PNH testing. **Recommendations:** PNH testing should be considered for patients with unexplained hemoglobinuria, Coombs-negative hemolytic anemia, thrombosis of unusual sites (including hepatic and cerebral vessels), synchronous thrombosis and cytopenias, myelodysplastic syndromes with unilineage dysplasia, and aplastic anemia. PNH testing should be performed on peripheral blood, ideally but not necessarily in EDTA and within 48 hours of collection. Granulocyte and monocyte analysis using a stain-then-lyse protocol is most accurate for measuring GPI-deficient cells. Light scatter and CD15 (bright) back-gating are ideal for granulocyte gating; CD33/CD64 or CD163 is ideal for back-gating for monocytes. Fluorescent aerolysin (FLAER) binds specifically to GPI in a maturation-independent manner and is the best PNH reagent, ideally in combination with CD24 or CD66b for granulocytes and CD14 for monocytes. CD16a is less optimal. CD55 and CD59 are not recommended. Red cell analysis must be performed in cases with leukocyte GPI deficiency and may be performed in all cases, ideally with a stain-then-wash protocol that employs multiple washes and vigorous mechanical disaggregation (vortexing, "racking"). Glycophorin A back-gating is used with CD59 staining to distinguish and quantify normal (Type I), partially deficient (Type II), and GPI-deficient (Type III) erythrocytes. Pathologic erythrocytes typically number less than GPI-deficient leukocytes. Reporting of large PNH clones should include the percentage of GPI-deficient cells for granulocytes, monocytes, and red cells. Small PNH-like populations (<1%) should also be described, especially in myelodysplastic syndrome and aplastic anemia patients, since these indicate a need for follow-up monitoring, regardless of whether they represent truly clonal populations.

**Conclusions:** Routine diagnosis and monitoring of PNH can be achieved by careful and deliberate attention to standard clinical flow cytometric practices as outlined in this report.

**Reviewer’s Comments:** Laboratories can apply current guidelines to effectively establish routine, diagnostic assays for clinically significant disease (as low as 1% GPI-deficient cells). High-sensitivity assays (as low as 0.01% GPI-deficient cells) require acquisition of many events (250,000 to 500,000 cells of interest) and substantial laboratory experience with running and interpreting the assay. Monitoring of PNH patients treated with eculizumab will reveal paradoxical post-treatment increases in the fraction of GPI-deficient red cells, since abnormal red cells are now protected from complement-mediated hemolysis. (Reviewer-Guy E. Nichols, MD, PhD).

**Keywords:** Paroxysmal Nocturnal Hemoglobinuria, Flow Cytometry, FLAER, Eculizumab, CD24
European consensus polymerase chain reaction primer sets recommended for standardization of B- and T-cell clonality assays showed similar or worse sensitivity compared to a conventional T-cell receptor-gamma assay and slightly lower specificity.

**Background:** In 2003, an extensive European collaborative effort developed a standardized multi-plexed PCR primer sets and protocols for optimal evaluation of B- and T-cell clonality (immunoglobulin and T-cell receptor [TCR] gene rearrangement). The goal was to improve and standardize PCR clonality assays as adjunct tests in the diagnosis of lymphoma and leukemia. These primer sets and protocols have been validated in frozen tissue specimens but not in archival paraffin-embedded tissues.

**Objective:** To apply recently standardized PCR primer sets and protocols (Biomed-2 Concerted Action) to analysis of T-cell clonality in archival formalin-fixed paraffin-embedded skin tissues.

**Methods:** 107 consecutive formalin-fixed paraffin-embedded skin lesions were retrieved from archives in the Benjamin Franklin, Charité-Universitätsmedizin Berlin pathology department. These lesions included 84 cutaneous T-cell lymphomas, 3 systemic T-cell lymphomas, and 20 controls (5 B-cell lymphomas, 13 benign dermatoses, and 2 pseudolymphomas). Extracted DNA was amplified using 2 TCR-gamma PCR (Biomed sets A and B) and 3 TCR-beta PCR (Biomed sets A, B, C) and was compared to an in-house TCR-gamma assay.

**Results:** Among 84 paraffin-embedded skin lymphomas, the Biomed TCR-gamma assay demonstrated clonal PCR products in 68 samples (81%) compared to 72 (86%) for the in-house TCR-gamma assay. The more complex Biomed TCR-beta assay was unsuccessful in 4 cases and demonstrated clonal PCR products in 62 of 80 samples (78%). All 3 assays were statistically comparable. Combining the Biomed TCR-beta and -gamma assays increased the sensitivity to 87%. The in-house TCR-gamma assay was 100% specific, but the Biomed TCR-beta and -gamma assays together produced 3 equivocal results in the control set.

**Conclusions:** Biomed-2 consensus multi-plexed PCR primer sets and protocols are recommended for standardization of B- and T-cell clonality assays but, in 84 paraffin-embedded cutaneous T-cell lymphomas, showed comparable sensitivity to a conventional TCR-gamma assay with slightly lower specificity.

**Reviewer's Comments:** These results are not going to compel an established molecular diagnostic lab to exchange its existing TCR-gamma primer sets or protocols. In my experience, specificity is a major concern for T-cell clonality PCR assays. Due to the limited repertoire of TCR-gamma rearrangements, occasional benign reactive oligoclonal T-cell populations can be amplified, producing "pseudoclonal" results, and this risk of false-positive results increases with limited sample size. Most successful laboratories may prefer their current TCR-gamma protocols but may consider adding a TCR-beta assay. (Reviewer-Guy E. Nichols, MD, PhD).

**Keywords:** Cutaneous T-Cell Lymphoma, Biomed-2 Polymerase Chain Reaction

**Print Tag:** Refer to original journal article
Well-differentiated osteosarcomas of the jaw are very rare and have a good prognosis.

**Background:** Osteosarcoma of the jaw bones is somewhat uncommon. The site accounts for approximately 6% of skeletal osteosarcomas. Most of the tumors arise in the mandible, usually in the third or fourth decade of life. Most tumors are high-grade; lower-grade tumors (such as parosteal osteosarcomas) are very rare. As such, intramedullary osteosarcomas are sometimes mistaken for fibro-osseous lesions, whereas extramedullary tumors can be confused for osteomas.

**Objective:** To describe a series of well-differentiated gnathic osteosarcomas.

**Methods:** The surgical pathology files of a single institution as well as the authors' personal consultation files were searched for all cases of gnathic, grade 1 of 3 osteosarcomas. Histology and radiographs (when available) were reviewed. Clinical information and follow-up were pursued.

**Results:** There were 15 cases from 8 females and 7 males. More than half the patients were aged >40 years. Nine tumors arose in the maxilla, and 6 were in the mandible. No patient had a history of radiation exposure, and all tumors except 1 were intramedullary. The intraosseous tumors were variable radiolucent and dense and were poorly defined. Tumors eroded through the cortex into the soft tissue with an associated periosteal reaction or expanded the bone without cortical destruction. The tumor that arose on the surface of the bone merged with the underlying bone and had an appearance similar to that of an osteoma. Tumors were white-tan and hard, and sizes ranged from 0.5 to 6 cm. The parosteal osteosarcoma was comprised of thick trabeculae of woven bone with surrounding fascicles of minimally atypical spindled cells without mitotic activity. The tumor infiltrated the underlying cortex and was otherwise surrounded by a pseudocapsule. The intramedullary tumors were hypo-cellular to moderately cellular, with uniform mildly atypical spindled cells arranged in intersecting fascicles with variable amounts of collagen. Mitotic activity was very low. Most bone formation was woven and trabecular. In nearly half the cases, neoplastic cartilage was present. Wide excision was performed in most cases, and a few cases had marginal resections with positive margins. No cases developed recurrences or metastases.

**Conclusions:** Gnathic osteosarcomas are infrequent, are predominately intramedullary, and have an excellent prognosis.

**Reviewer's Comments:** Pathologists should keep low-grade osteosarcomas in mind before signing out gnathic fibro-osseous lesions. Correlation with radiographic findings is important. (Reviewer—Edward B. Stelow, MD).

**Keywords:** Osteosarcoma, Gnathic, Well Differentiated, Mandible

**Print Tag:** Refer to original journal article
New Clear Cell Renal Cell Carcinoma Described

Clear Cell Tubulopapillary Renal Cell Carcinoma: A Study of 36 Distinctive Low-Grade Epithelial Tumors of the Kidney.

Aydin H, Chen L, et al:


The renal cell carcinoma described in this manuscript appears to be distinct from conventional clear cell and papillary renal cell carcinomas.

Background: Since the publication of the 2004 World Health Organization classification of renal tumors, a number of new, low-grade renal tumors have been described. Publications have described low-grade clear cell carcinomas with a papillary architecture that were immunoreactive with antibodies to CK7 and non-reactive with antibodies to CD10. None of these have had cytogenetic abnormalities typically seen with clear cell or papillary renal cell carcinomas, and none arose in patients with von Hippel Lindau (VHL) disease.

Objective: To review a series of renal tumors similar to these that the authors have termed "clear cell tubulopapillary renal cell carcinomas."

Methods: Tumors were seen over almost a 20-year period. Demographic, clinical, and gross information was collected. Microscopic features were reviewed. Immunohistochemistry was performed with a number of antibodies. FISH was performed to detect abnormalities of chromosomes 3p, 7, and 17. The VHL gene was sequenced.

Results: There were 36 tumors from 33 patients. Of consecutive cases, these represented <1% of renal tumors. The tumors were from 17 women and 16 men, ranging in age from 26 to 88 years. Three patients had bilateral tumors, and one had VHL Disease. Most patients underwent partial nephrectomy only. Only one of 20 patients with follow-up had any evidence of disease; this was a patient with VHL disease who had a contralateral renal tumor. Most tumors were grossly cystic, and the mean size of the tumors was 2.4 cm. Microscopically, most tumors were multicystic and were surrounded by a thick capsule. Most tumors also had prominent fibrotic stroma with tumor cells embedded in the stroma. Smooth muscle fibers by immunohistochemistry in most cases. Cysts frequently had short papillae within them, and nearly all tumors had areas of tubular or acinar formation. Papillae were present in the majority of cases, as were small nests of clear cells. Most tumors showed multiple growth patterns. Cells had scant eosinophilic cytoplasm or a moderate amount of clear cytoplasm. Intraluminal proteinaceous secretions were present. Nuclei were often located away from the basal portions of the cells. Foamy histiocytes were not present. Nearly all cases were pT1a tumors. Approximately half were Fuhrman grade 1, and half were grade 2. All tumors were CK7 immunoreactive, diffusely and strongly positive. Nearly all cases were immunoreactive with antibodies to CA9. Most tumors were non-reactive with antibodies to CD10. When immunoreactive, CD10 was present in the minority of cells. By FISH analysis, only one case tested showed low-copy number gain of chromosome 7 and 17. No 3p losses or VHL mutations were seen.

Conclusions: Clear cell tubulopapillary renal cell carcinoma appears to be a unique variant of renal cell carcinoma that has an excellent prognosis.

Reviewer's Comments: Our understanding of the diversity of renal cell carcinoma continues to expand. Hopefully, the tumor described here will be reproducibly recognized. (Reviewer-Edward B. Stelow, MD).

Keywords: Renal Cell Carcinoma, Clear Cell, Papillary, Immunohistochemistry

Print Tag: Refer to original journal article