Transfusion-transmitted *B. microti* is a significant cause of transfusion-related mortality.

**Background:** *Babesia microti*, a tick-borne parasite endemic to the northeast and upper Midwest, is the primary cause of human babesiosis in the United States. Although these infections are usually subclinical or self-limited in immunocompetent people, severe disease can be present in susceptible populations (including the elderly or immunocompromised). Here, infections result in hemolytic anemia, thrombocytopenia, hematuria, and renal failure. The mortality rate has been reported to be approximately 5% in such populations. The parasite is located inside red blood cells (RBCs) and classically is identified by a maltese cross formation of the parasites on blood smear preparation. A chronic carrier state can be present in some patients, and, in fact, *B. microti* is the most frequently transmitted parasitic agent in blood transfusion in this country. Importantly, the parasite remains viable in RBCs for 35 days at 4°C and survives in cryopreserved RBCs.

**Objective:** To describe 18 cases of transfusion-transmitted *B. microti* between 2005 and 2007.

**Methods:** All cases were identified by the American Red Cross hemovigilance program (ARC HP). Follow-up samples from the donors were tested using an immunofluorescent assay or with real-time polymerase chain reaction (PCR).

**Results:** From an initial 32 suspected cases, 18 cases were classified as definite or probably transfusion-transmitted babesiosis. Most recipients were between the ages of 61 and 84 years, while 2 recipients were <2-years-old. Four recipients were asplenic. Five recipients (28%) died within a short period of time after being diagnosed (days to weeks); all 5 had significant preexisting medical conditions. Regarding the donors, 65% were residents of endemic areas (Connecticut, New Jersey, and Massachusetts), and 4 other donors had traveled to endemic areas. In follow-up testing, only 1 of 12 samples tested by PCR was positive, but all donors were positive for antibodies against *B. microti*. None of the donors recalled symptoms around the time of donation, but some (3 donors) had a recollection of tick bites.

**Conclusions:** Transfusion-transmitted babesiosis is a significant and increasing issue in blood safety. This is highlighted by numerous articles on this topic in the December 2009 edition of *Transfusion*. The incidence has been estimated at 1 case in 1.1 million RBC units, although this is likely an underestimation. Currently, there is only 1 question on the donor health history questionnaire asking if the donor has a history of babesiosis. Unfortunately, there is no Food and Drug Administration-approved test for *B. microti*.

**Reviewer's Comments:** This article highlights the need for interventions to reduce patient risk of transfusion-transmitted *B. microti*. As of now, only directed screening through questionnaires is the best approach to identify at-risk donors. (Reviewer-William A. Kanner, MD).

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**Keywords:** Blood Transfusion, *Babesia microti*

**Print Tag:** Refer to original journal article
**Background:** Fecal occult blood testing (FOBT) has been used for decades to screen for colorectal carcinoma. The traditional guaiac FOBT suffers from some limitations, including cross reactivity and reproducibility. Therefore, immunochemical FOBTs (IFOBTs), which can be automated, have been developed to specifically identify human hemoglobin (Hb) in stool specimens.

**Design/Objective:** In this large, prospective study, the authors evaluate the utility of IFOBTs at various Hb thresholds in screening for colorectal carcinoma.

**Methods:** Consecutive, asymptomatic or mildly symptomatic ambulatory patients from 3 endoscopy centers, who were scheduled to have colonoscopy for screening purposes, were prospectively included in the study. Patients with known rectal bleeding or incomplete colonoscopy examination results were excluded. The participants received instructions to prepare 3 consecutive IFOBTs the week before colonoscopy without diet or medication limitations. Samples were stored at 4ºC and processed within 3 weeks. The IFOBTs were processed on the OC-MICRO instrument and Hb levels were recorded; <50 ng Hb/mL was considered negative. At colonoscopy, any lesions identified were biopsied. Adenomatous polyps (APs) were considered advanced if they were >10 mm, >20% villous, or contained high-grade dysplasia.

**Results:** 1682 ambulatory patients completed both IFOBT and colonoscopy screening. Colonoscopy detected colorectal carcinoma in 20 patients and advanced APs in an additional 129 patients. Using the lowest possible threshold (>50 ng Hb/mL) on any of 3 IFOBTs, the sensitivity and specificity for detecting colorectal carcinoma were 100% and 85%, respectively. The sensitivity and specificity for detecting advanced APs were 55% and 88%, respectively. All cancers, but only half of advanced APs, were detected at the lowest threshold. Average-risk screening tests for colorectal carcinoma are recommended to aim for >95% specificity; this could be achieved by using 1 IFOBT test with a threshold of 100 ng Hb/mL (sensitivity of 65% for carcinoma and 26% for advanced APs). Using the most sensitive threshold and 3 tests would result in 3 patients undergoing colonoscopy for every carcinoma or advanced AP detected; 1 test at the 100 ng Hb/mL threshold would result in 2 patients undergoing colonoscopy for every carcinoma or advanced AP detected.

**Conclusions:** IFOBTs can detect most colorectal carcinomas and advanced APs. The screening policy chosen will determine test sensitivity and specificity.

**Reviewer’s Comments:** This well-designed study demonstrates the importance of setting appropriate thresholds for various screening purposes; in high-risk patients, 3 IFOBTs with a low threshold is appropriate, while in average-risk patients, 1 IFOBT with a cutoff at 100 is more appropriate. Recommending appropriate testing for our clinical colleagues will vary according to the individual institution's patient population. (Reviewer-Deborah J. Chute, MD).

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Keywords: Adenoma, Colorectal Ca, IFOBT, Predictive Values, Sensitivity, Specificity

Print Tag: Refer to original journal article
Physiological Hepatocyte Nuclear Vacuolation Persists Beyond Young Adulthood

Physiological Hepatocyte Nuclear Vacuolation—How Long Does It Persist?

Levene AP, Goldin RD:

Histopathology 2010; 56 (March): 426-429

Hepatocyte nuclear vacuolation normally persists into and beyond young adulthood; vacuolation not associated with NAFLD appears to decrease with age and vacuolation associated with NAFLD appears to increase with age.

Background: Vacuolation of hepatic nuclei may be either physiological or pathological. Pathological causes include obesity, diabetes, Wilson's disease, and glycogen storage diseases. While it is recognized that hepatocyte nuclear vacuolation is more commonly seen in childhood and adolescence, the length of time that this change persists has not been well defined. The presence of prominent hepatocyte nuclear vacuolation is thought to more strongly support non-alcoholic fatty liver disease (NAFLD), including non-alcoholic steatohepatitis (NASH), than fatty liver disease caused by alcohol. Thus, there might be value in knowing how long nuclear vacuolation normally persists in the liver in order to better understand the potential significance of this finding, especially in younger patients with fatty liver disease.

Objectives: To determine how long hepatocyte nuclear vacuolation persists and to define how this may help in the interpretation of liver biopsy specimens in the setting of possible NAFLD.

Methods: 872 liver biopsies from patients with chronic hepatitis B were selected. Patients with hepatitis B were chosen since this disease has no known association with hepatocyte nuclear vacuolation. Clinical risk factors for fatty liver disease were defined as an elevated body mass index (BMI) (>25 kg/m2) and alcohol intake of >15 units/week. Pre-enrollment clinical screening and laboratory testing confirmed that each patient lacked these clinical risk factors, and that they had no biochemical evidence of copper overload, iron overload, or α1-antitrypsin deficiency. Patients also tested negative for human immunodeficiency virus infection and hepatitis C virus infection. One pathologist, who was blinded to clinical and demographic information, examined all biopsies and counted the total number of hepatocytes as well as the number of hepatocytes with nuclear vacuolation in each specimen. Signs of NAFLD, including steatosis and NASH, were also recorded.

Results: 40 of the 872 cases showed nuclear vacuolation. Although most common within the youngest age group studied (20 to 24 years of age), nuclear vacuolation was seen in all age groups in the absence of clinical risk factors for fatty liver disease. Nuclear vacuolation was present in <5% of hepatocytes. No significant correlation was found between the number of vacuolated nuclei and the presence/absence of steatosis. Vacuolation not associated with NAFLD was observed in 13% of 20- to 24-year-olds and in 0.5% of 40- to 64-year-olds (P <0.01). Vacuolation associated with NAFLD was observed in 1.9% of 20- to 24-year-olds and in 6% of 60- to 64-year-olds.

Conclusions: Physiological hepatocyte nuclear vacuolation persists beyond young adulthood. Nuclear vacuolation not associated with NAFLD appears to decrease as patients get older, while vacuolation associated with NAFLD appears to increase with age.

Reviewer's Comments: The observations underscore the "soft" nature of using this finding to support an NAFLD diagnosis. It would have been helpful to include younger patients in the study. (Reviewer-T. David Bourne, MD).

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Keywords: Liver, Hepatocyte Nuclear Vacuolation

Print Tag: Refer to original journal article
Chronic colitis may sometimes be associated with NSAID use.

**Background:** The diagnosis of "chronic colitis" implies a state of chronic injury to the colon. It is characterized by crypt architectural distortion and a basal plasmacytosis. The histologic pattern is usually seen in patients with idiopathic inflammatory bowel disease. Occasionally, the changes may be seen with long-standing colonic infection, chronic ischemia, immunodeficiency states, and drug-induced colitis. The clinical significance of the finding of chronic colitis in a patient who is asymptomatic and does not have a history of idiopathic inflammatory bowel disease is unknown.

**Objective:** To report the clinical follow-up on a series of patients found to have asymptomatic chronic colitis.

**Methods:** A single institutions surgical pathology database was searched for all cases of adult onset chronic colitis seen over an 8-year period. Patients who had previously diagnosed inflammatory bowel disease were excluded. Patients with known ischemic, pseudomembranous, or granulomatous colitis were excluded as were patients with an immunodeficiency disorder. If the biopsies were taken adjacent to polyps, the case was also excluded. The degrees of activity and chronicity were scored based on histologic features. Clinical, demographic, endoscopic, and follow-up data were pursued.

**Results:** 17 cases of chronic colitis were identified that did not have a recognized cause. Sixteen cases involved the cecum or ascending colon. Ten of the patients were men, and the mean age was 59 years. The majority of patients was asymptomatic or showed minimal symptoms. No patients had acute diarrhea. The epithelium appeared endoscopically abnormal in the majority of cases, with the most common abnormality being erythema. Recent nonsteroidal anti-inflammatory drug (NSAID) use was noted in 8 of the patients. Seven patients were receiving atorvastatin. Chronic changes were present only focally in 6 of the cases. Crypt architectural distortion was seen in all cases, and 16 had obvious basal plasmacytosis. At least mild activity was seen in 95% of cases, and crypt abscesses were seen in 65% of cases. Intraepithelial lymphocytes and collagen deposition were not seen. No patients developed inflammatory bowel disease in an average follow-up time of 43 months. Of the 5 patients with follow-up biopsies, lesions resolved in the 2 for whom NSAID therapy was discontinued. In the other 3 cases, the patients continued their NSAID use, and the histologic changes remained unchanged.

**Conclusions:** NSAID use may account for some cases of chronic colitis seen in patients without other known risk factors for the findings. Pathologists should be aware of this so as to not erroneously diagnose idiopathic inflammatory bowel disease.

**Reviewer's Comments:** It is interesting that in this study of asymptomatic chronic colitis, most patients had activity. This confirms the fact that chronic colitis results from chronically active disease. (Reviewer-Edward B. Stelow, MD).

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Keywords: Colitis, Chronic, NSAID

Print Tag: Refer to original journal article
As in cervical dysplasia, AIN associated with HPV genotypes 16 and 18 are more likely to progress than those associated with other genotypes.

**Background:** Anal carcinoma is highly associated with human papillomavirus (HPV), especially the high-risk genotype 16. Although historically an uncommon malignancy, the incidence is rising in transplant recipients and HIV-positive patients, in particular. Anal carcinoma is similar to cervical carcinoma by having a precursor lesion referred to as anal intraepithelial neoplasia (AIN). Comparable to the cervix, low-grade dysplasias are likely to regress in immunocompetent patients, but may progress to higher grade lesions in patients with a compromised immune status. Many studies on the HPV genotypes of AIN have been performed on an anal swab rather than the corresponding tissue biopsy. This may overestimate associations since multiple HPV infections are common in this scenario, all of which may be sampled by a swab, but not all of which are responsible for histologically identified AIN.

**Objective:** To genotype HPV in tissue biopsies of AIN and anal carcinoma to characterize lesion-specific infections and associate the genotype with clinical course.

**Methods:** Archived tissue was collected to represent anal biopsies and resections of varying normal AIN and anal carcinomas. These were reviewed for concordant diagnosis and submitted for high-risk HPV testing via polymerase chain reaction (PCR), subtyped by nesting PCR or the Invader Assay. The clinical course was documented for comparison.

**Results:** High-risk HPV (HR HPV) genotypes were detected in 40 of 53 (75%) AIN cases: 24 of these were HPV 16, 3 were HPV 18, and 14 were other high-risk types (non-16/18). Of the immunocompromised patients, 69% were positive for HR HPV types, and of the immunocompetent patients, 83% were positive. HPV 16 was the most common in both populations. HR HPV detection was directly correlated with degree of dysplasia (0% in normal, 56% in AIN I, 80% in AIN II, 88% in AIN III, and 89% in squamous cell carcinomas). Twenty-four percent of the lesions were in women, and only 35% were in patients documented to be immunodeficient. Two of 10 anal adenocarcinomas were positive for HR HPV. Progression was noted on follow-up of 3 cases, which were all immunodeficient and all associated with HPV 16 or 18. Persistence was noted in 9 cases and regression in 14; most were immunocompetent and associated with variable HPV genotypes.

**Conclusions:** AIN and anal squamous carcinomas are highly associated with HR HPV, most notable HPV 16 and to a lesser extent HPV 18. Lesions associated with HPV 16/18 are more likely to progress than those with non-16/18 HPV types.

**Reviewer’s Comments:** Last year, the Food and Drug Administration approved the use of an HPV vaccine in boys that could alter the characteristics of this male-prevalent disease. Clinicians should be aware that women and immunocompetent patients do acquire AIN. (Reviewer-Mary T. Galgano, MD).

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Keywords: HPV-Related Neoplasia

Print Tag: Refer to original journal article
It is essential to be aware of the various activities that contribute to musculoskeletal pain and disorders.

Background: There is a high prevalence of musculoskeletal disorders among pathologists, mainly due to microscope and computer use under less than optimal ergonomic conditions. Excessive force, repetitive movements, vibration, and awkward or prolonged static posture are all associated with these disorders and injuries. These factors lead to a persistent and recurrent cycle of inflammation and tissue injury with fibrosis resulting in pain and dysfunction of muscles and ligaments. There is also research to suggest that there are increased levels of endogenous substances that accumulate and interfere with neuromuscular function and pain pathways.

Objective: To discuss occupational hazards for pathologists, focusing on neck and upper back pain. Work-Related Cumulative Trauma: Although poor or awkward posture at the microscope is an immediate culprit, we may not realize that high levels of static contraction and prolonged static load lead to increased risk of musculoskeletal disorders. Postulated explanations for the association between static loading and injury include local ischemic injury, local disturbances of metabolites, and aberrant contraction-rest cycles in small muscle fibers. Many of these factors can be attributed to the fact that the neck and back muscles are truly at work even while we are sitting down viewing slides or using the computer. Microscopes with tilting and telescoping eyepieces are preferred. However, even with these new microscopes, there is still a tendency to lean forward if the microscope is not correctly positioned on the table. Further complicating things is the combination of microscope and computer work, especially with those who wear corrective lenses.

Management may include a worksite evaluation by someone with experience in ergonomics, anti-inflammatory medicine, and physical and/or massage therapy. Centers for Disease Control and Prevention (CDC) Guideline for Microscope Use: These have been modified by Dr. George, and the key points are summarized in following sentences. A suitable workstation design is essential, and ideally, it should include an adjustable chair, work surface, and microscope. Also, one must take proper rest. The CDC recommends no more than 5 hours a day of microscope use. Exercise is also an important component and should focus on stretching exercises involving the back, neck, arms, wrists, and hands. Fourthly, avoid forward leaning that disrupts the neutral spinal posture. Important adjustments to aid in this include microscope placement, ample room under the table or using a cutout work table, and a microscope with adjustable eyepieces. Finally, an ergonomically designed chair provides proper upper and lower back support.

Reviewer’s Comments: In our line of work, we are all prone to musculoskeletal pain, and I have observed many colleagues with such complaints. Therefore, prevention strategies are instrumental to a healthy career. (Reviewer-Stacey E. Mills, MD).

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Keywords: Ergonomics, Microscope, Musculoskeletal Disorders

Print Tag: Refer to original journal article
FNA of Distal Extremities Helps Diagnose Benign, Malignant Lesions

Jakowski JD, Mayerson J, Wakely PE Jr:

Am J Clin Pathol 2010; 133 (February): 224-231

FNA of the distal extremities is a clinically reliable procedure and appears to be useful in the diagnosis and management triage of benign and malignant lesions.

**Background:** Fine-needle aspiration (FNA) is a clinically useful tool for evaluating superficial and deep lesions in many body sites. However, there is little information in the literature regarding FNA of the distal extremities. Evaluation is often limited to the clinical features and radiologic findings.

**Objective:** To review the authors experience with FNA of the upper and lower extremities.

**Methods:** The data span 11 years and include only cases with tissue biopsy confirmation or a minimum 1-year follow-up. Most FNAs (21- or 22-gauge needles, subcutaneous route) were performed after a review of the images. An initial assessment was completed within 20 minutes using a Romanowsky stain. Cell blocks were attempted when feasible, and flow cytometry was sent for if indicated.

**Results:** Of the 141 cases identified, 41, 23, 34, and 43 FNAs were from the hand, wrist, ankle, and foot, respectively. Benign lesions greatly outnumbered malignant lesions (110 vs 31). The wrist was the least common area for FNA but had the highest overall malignant diagnoses, while the hand had the lowest percentage of malignancies. Most FNAs of the hand were from the palmar surface and the fingers. Approximately 50% of the aspirates of the foot were from the toes. Most malignant FNAs were from the dorsum of the hands and feet, while most benign FNAs involved the fingers and toes. A curious finding was that malignant diagnoses were 8 times more likely on the right foot. Of the cases, approximately 72% had a specific diagnosis, 26% had a descriptive diagnosis, and 2% had a suspicious-for-malignancy diagnosis. Of the 34 malignant or suspicious-for-malignancy diagnoses, 4 were false positives (2 resulted in a diagnosis of nodular fasciitis, 1 pigmented villonodular synovitis, and 1 synovial hemangioma). Importantly, in all 4 cases, there was not enough material for a cell block. The great majority of malignancies were sarcomas. Of the benign diagnoses, 44% of the specific diagnoses and 57% of the descriptive benign diagnoses underwent a diagnostic excision or biopsy. Ganglion and giant cell tumor of the tendon sheath were the 2 most common diagnoses. Only 2.8% of cases were infectious. The overall sensitivity and specificity in distinguishing a benign from a malignant lesion was 100% and 96%, respectively, while the positive and negative predictive values were 88% and 100%, respectively.

**Conclusions:** FNA of the distal extremities is clinically useful in the diagnosis and management triage of benign and malignant lesions, even considering that there are >100 distinct histopathologic tumor types in these locations.

**Reviewer's Comments:** The authors do note that there is a patient population bias from an orthopedic oncology clinic, and these cases do not include skin lesions. The authors also emphasize that nodular fasciitis is a common pitfall in aspiration cytology. (Reviewer-William A. Kanner, MD).

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Keywords: FNA, Wrist, Hand, Foot, Ankle

Print Tag: Refer to original journal article
Using the most recent criteria for thyroid FNA adequacy, the unsatisfactory rate dropped from 19% to 13%, without a loss of specificity.

Background: Thyroid fine-needle aspiration (FNA) is a screening test designed to reduce unnecessary surgery and direct further management in patients with thyroid nodules. A major limitation of this technique is the skill of the aspirator in performing the procedure and slide preparation. Criteria used to determine adequacy of thyroid FNAs has been somewhat controversial.

Objective: To examine the impact of reclassifying thyroid FNAs previously considered unsatisfactory with recently proposed criteria of adequacy.

Methods: All thyroid FNAs over a 15-year period with a diagnosis of "inadequate for evaluation" and subsequent follow-up histology or cytology results were included for evaluation. All smears were reviewed by 2 independent cytopathologists. FNAs were considered adequate if at least 6 groups of 10 follicular cells or at least 60 total follicular cells were present, discernible, and viable. Aspirates with poor quality smears (due to excess blood, thick smears or poor cellular preservation) were considered inadequate. When adequate, FNA smears were divided into the following categories: non-neoplastic; follicular lesion; suspicious for malignancy; and diagnostic of malignancy. Follow-up results were compared with FNA results.

Results: 18.8% of all thyroid FNAs were originally called inadequate; 279 of these had follow-up results. Using the above criteria, 82 FNAs (29%) were considered adequate on review. Of these, 66 (80%) were classified as non-neoplastic on FNA, 14 (17%) were classified as follicular lesion, and 2 (3%) were classified as suspicious for malignancy. On follow-up, 5 cases (6%) were malignant (2 lymphomas, 1 papillary thyroid carcinoma, 1 medullary carcinoma, and 1 metastatic squamous cell carcinoma). The specificity of a non-neoplastic diagnosis was 94%. Two malignant cases were classified as adequate and non-neoplastic on repeat review; both were <1 cm in size and likely represented sampling error. In the 197 cases that remained inadequate, the most frequent cause was limited cellularity (82%), with poor slide preparation being the second most frequent cause (13%). On follow-up of the inadequate FNAs, 5% were malignant.

Conclusions: Using the recently proposed criteria for thyroid FNA adequacy, 29% of thyroid FNAs were reclassified as adequate. This resulted in a drop of the unsatisfactory rate from 18.8% to 13.2%. There was no significant loss of specificity in the low-cellularity cases reclassified as adequate and non-neoplastic.

Reviewer's Comments: This study nicely demonstrates that the proposed criteria for thyroid FNA adequacy are realistic and will not result in an increased number of false-negative diagnoses. When this group included abundant colloid with limited follicular cells as adequate, their unsatisfactory rate dropped to 12%. (Reviewer- Deborah J. Chute, MD).

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Keywords: Thyroid, FNA, Adequacy

Print Tag: Refer to original journal article
New Method to Screen for Cystic Fibrosis

Newborn Screening for Cystic Fibrosis by Use of a Multiplex Immunoassay.

Lindau-Shepard BA, Pass KA:

Clin Chem 2010; 56 (March): 445-450

A newly developed multiplex immunoassay for CF screening shows equivalent sensitivity and probable increased specificity compared with current blood immunoreactive trypsinogen tests.

Background: Cystic fibrosis (CF) is the most common fatal autosomal recessive disease among Caucasians, and its disease incidence is approximately 1 in every 2000 to 3000 live births. Currently, 46 U.S. states have newborn screening (NBS) programs for CF, all of which utilize tests to detect blood immunoreactive trypsinogen (IRT). IRT concentrations are elevated in newborns with CF. Of the 2 proenzymes secreted by pancreatic exocrine cells (cationic trypsinogen or IRT1 and anionic trypsinogen or IRT2), IRT1 levels are higher than IRT2 levels in the absence of disease. However, IRT2 concentrations tend to be higher than IRT1 in disease states. Current NBS programs consist of an initial screening test followed by confirmation by repeat testing on a new specimen or testing with a different methodology, such as DNA analysis. Unfortunately, screen-positives to confirm CF case ratios of 30:1 are not unusual. Thus, screening methodologies offering greater specificity, increased standardization, and the potential for external quality control (QC) are needed.

Objectives: To report the development of a multiplexed immunoassay for the detection of the 2 main trypsinogen isoforms (IRT1 and IRT2) and to compare its test characteristics with a commonly used CF kit using standard IRT testing.

Methods: Immunoassays were created by coupling antitrypsin isoform-specific monoclonal antibodies to Luminex beads. Assays were developed for each isoform individually, followed by development of a multiplexed assay based on combined IRT1 and IRT2. Samples consisted of specimens from the New York State Department of Health NBS program. Initial screening consisted of single sample analysis using the Blood Spot Trypsin MW ELISA by MP Biomedicals. Comparison analysis between the standard method and the multiplex immunoassay was performed on 168 blood spot samples.

Results: Results of the multiplex assay, which used summed assay values (IRT1 + IRT2), were comparable to the standard IRT test (correlation coefficient =0.75). Of the 35 samples that were screen-positive by the standard IRT test, 11 were screen-negative using the multiplex assay. Mutation analysis showed that these 11 cases were, in fact, negative for CF.

Conclusions: A multiplex immunoassay based on combined detection of IRT1 and IRT2 has screening performance comparable to that of standard IRT test methods in terms of sensitivity. The multiplex assay, however, appears to show greater specificity for CF. Furthermore, the multiplex design may allow for even greater increases in specificity with the addition of other biomarkers, such as pancreatitis-associated protein. Improved standardization may also be possible.

Reviewer’s Comments: The authors have experience with the development and implementation of multiplex immunoassay testing as applied to newborn screening. Importantly, the authors emphasized in their discussion that the multiplex assay, as designed in this study, cannot reliably distinguish CF carriers from patients with the disease. (Reviewer-T. David Bourne, MD).

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Keywords: Cystic Fibrosis, Newborns, Screening, Multiplex Immunoassay

Print Tag: Refer to original journal article
Benign smooth muscle tumors of the bladder can usually be diagnosed by transurethral resection.

**Background:** Mesenchymal tumors of the bladder are rare. Smooth muscle tumors are the most common benign and malignant mesenchymal tumors to be found at the site. Typically, leiomyosarcomas are those smooth muscle tumors with any degree of mitotic activity, atypia, or increased cellularity.

**Objective:** To examine a large series of bladder smooth muscle tumors to: identify features that would assist in distinguishing the tumors from normal bladder muscularis propria on transurethral resections; determine whether some degree of cellular atypia can be seen with benign bladder leiomyomas; determine the degree of heterogeneity that can be seen throughout individual tumors; and determine whether a smooth muscle tumor transurethrally resected can be definitively diagnosed as benign.

**Methods:** 51 smooth muscle tumors of the bladder seen at 3 institutions were reviewed. Twenty cases had been previously diagnosed as malignant. Circumscription, degree of atypia, mitotic activity and cellularity, epithelioid features, tumor cell necrosis, hyalinization, and myxoid changes were recorded. Leiomyosarcomas were classified as low and high grade. Follow-up information was pursued.

**Results:** The mean age of patients with leiomyomas was 52 years, and for patients with leiomyosarcomas, the mean age was 58 years. Patients with leiomyosarcomas were more likely to be men. Specimens consisted primarily of transurethral resections. The mean size was 4.5 cm for leiomyomas and 4.9 cm for leiomyosarcomas. Leiomyomas were noninfiltrative and nodular, and they had large sheets of intersecting smooth muscle fascicles. No epithelioid features or increased cellularity was present. Focal hyalinization, myxoid change, fatty metaplasia, and necrosis were uncommonly seen. Focal, mild cytologic atypia was seen in 7 cases. No patients were ever diagnosed with malignant tumors at follow-up. One-quarter of leiomyosarcomas showed epithelioid features, and tumor cell necrosis was seen in 50% of the cases. The median mitotic count was 7.5 per 10 high-powered fields. Infiltration was typically seen as irregular nodules and with both low- and high-grade tumors. Only 1 tumor showed heterogeneity of grade throughout. No cases had areas that resembled leiomyomas. Patients with high-grade leiomyosarcomas had higher disease-related mortality than those with low-grade leiomyosarcomas (50% vs 0%).

**Conclusions:** Leiomyomas can be diagnosed with transurethral resection without risk of follow-up malignancy. Patients with high-grade leiomyosarcomas fare worse than those with low-grade sarcomas.

**Reviewer’s Comments:** This study consists of a large number of tumors that many of us will only rarely see. It is reassuring to see that smooth muscle tumors of the bladder can apparently be distinguished more easily than uterine smooth muscle tumors. (Reviewer-Earod B. Stelow, MD.)

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Keywords: Leiomyoma, Leiomyosarcoma, Histology, Follow-Up

Print Tag: Refer to original journal article
Clinicopathologic features of lung adenocarcinoma do not reliably predict mutational status of KRAS and EGFR. Since they are mutually exclusive, the more prevalent KRAS can be analyzed first, with EGFR second if necessary.

**Background:** Lung adenocarcinomas with EGFR mutations are associated with response to tyrosine kinase inhibitors (TKIs) and are notably prevalent in female never smokers, particularly those of Asian descent. They are also associated with a characteristic morphology, including micropapillary or nonmucinous bronchioloalveolar pattern with hobnail cells. Mutations in KRAS are also noted in a subpopulation of lung adenocarcinomas but are associated with a nonresponse to TKIs. EGFR and KRAS mutations are mutually exclusive, but there is no algorithmic approach to ordering mutational analysis in lung carcinomas.

**Objective:** To determine whether a clinicopathologic-based protocol can be established for mutational analysis.

**Methods:** 345 consecutive primary adenocarcinomas of the lung undergoing surgical resection were included for comparison. Each was subjected to mutational analysis for EGFR and KRAS and FISH for EGFR with correlation to clinicopathologic features.

**Results:** Mutational analysis stratified the patients as follows: 37 (11%) with EGFR mutations; 103 (30%) with KRAS mutations; and 205 (59%) negative for mutations in either and no tumor with co-existing mutations. Women and never-smokers were overrepresented in the EGFR+ tumors, as was a tumor having mild lymphocytic response and an absence of solid growth. Older patients and smokers were overrepresented in the KRAS+ tumors, as was a tumor having mucinous differentiation. FISH positivity (by Colorado criteria) of 344 tumors was found to be a significant predictor of EGFR positivity status. None of these features had the significance to be used as selection criteria for mutational analysis.

**Conclusions:** KRAS and EGFR mutational status in primary adenocarcinomas of the lung are not reliably predicted by clinicopathologic features. Given that the mutations are mutually exclusive, KRAS mutation should be performed first because of the higher prevalence in the Western population, with EGFR to follow if necessary.

**Reviewer's Comments:** The authors attempt to create an algorithmic approach to mutational testing in adenocarcinomas of the lung. Given that the mutations are mutually exclusive, one can follow the other unless initiation of therapy is urgently necessary. (Reviewer-Mary T. Galgano, MD).

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Keywords: Lung Adenocarcinoma, Mutational Analysis

Print Tag: Refer to original journal article
Younger women with ER-negative breast cancers are the most likely to benefit from contralateral prophylactic mastectomy.

**Background:** Although the efficacy of prophylactic mastectomy is known, especially for certain at-risk populations, the survival benefit for patients with breast cancer who undergo contralateral prophylactic mastectomy has not been well studied. In fact, a study examining the benefit of contralateral prophylactic mastectomy in BRCA1 and BRCA2 mutation carriers who had unilateral breast cancer failed to show a survival benefit after controlling for other prophylactic surgeries.

**Objective:** This study used a cancer registry to determine the possible survival benefit for patients who undergo contralateral prophylactic mastectomy.

**Methods:** Women within the SEER database treated surgically for unilateral breast cancer over a 6-year period were included. Patients with other cancers or without 2 years of follow-up data were excluded. Other exclusion criteria included age <18 years or >90 years, lack of histological confirmation, lack of tumor size data, and presence of distant metastases. Patients treated with only total mastectomy or modified radical mastectomy were included. Patients undergoing contralateral mastectomy were considered the study group, and those not undergoing contralateral mastectomy were considered the control group. Results were compared to outcomes.

**Results:** >107,000 patients met inclusion criteria. Of these, slightly more than 8% underwent contralateral prophylactic mastectomy. Patients who underwent contralateral prophylactic mastectomy were younger, had earlier stage disease, and were more likely to be white. Women who underwent contralateral prophylactic mastectomy with stage I to III disease had improved disease-specific survival by univariate analysis. Also according to univariate analysis, patient age, stage, grade, lymph node status, estrogen receptor (ER) status, race, and histology were associated with disease-specific survival. By multivariate analysis, contralateral prophylactic mastectomy, stage, grade, lymph node status, ER status, race, histology and age all remained statistically associated with survival outcome. Cancer-related survival for contralateral prophylactic mastectomy declined with increasing age, and patients aged >60 years had no improved survival. The greatest benefit was seen for patients <50 years old who had stage I or II ER-negative tumors. There was no association between contralateral prophylactic mastectomy and survival for patients with ductal carcinoma in situ.

**Conclusions:** Contralateral prophylactic mastectomy may be warranted in some younger patients with unilateral breast cancer. Although the survival benefit is small, it is greatest for those with low-stage, ER-negative tumors and is related to the risk of contralateral breast cancers in these patients.

**Reviewer's Comments:** This interesting study suggests that, for many patients with unilateral breast cancer, prophylactic contralateral mastectomy does not improve survival. Hopefully, these results will help surgeons counsel and treat these patients. (Reviewer-Edward B. Stelow, MD).

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Keywords: Cancer, Mastectomy, Prophylactic, Survival

Print Tag: Refer to original journal article
A group of melanocytic tumors may exist that are biologically different from conventional melanoma and benign nevi.

**Background:** There are particular entities that are difficult to distinguish from melanoma, particularly lesions with spindle and/or epithelioid cells. These entities include atypical Spitz tumors, atypical blue nevi, and the "deep penetrating nevi." Terms proposed for these lesions include melanocytic tumor of uncertain malignant potential (MELTUMP) or melanocytic proliferation with indeterminate biologic potential.

**Objective:** To study a large number of such tumors to determine if there are criteria for distinction between benign and malignant cases.

**Methods:** 59 cases were identified and reviewed by a panel. The participants initially reviewed the case individually (digital images), and then the panel convened at a meeting to discuss the cases. The cases were classified into 3 groups based on clinical behavior: favorable (no evidence of metastasis after follow-up of at least 5 years), unfavorable (tumor-related death and/or large metastatic deposits), and borderline (small deposits of tumor cells in lymph nodes). Histopathologic criteria evaluated included ulceration, symmetry, pagetoid spread, confluence of nests, maturation, atypia, mitoses, and inflammation among other categories.

**Results:** Of the 59 cases, there were slightly more females (33) than males, and the mean age was 29 years (range, 1 to 75 years). There was a fairly wide distribution of lesions including the trunk (18 cases), lower extremities (12 cases), upper extremities, shoulder, buttocks, foot, scalp, and face. According to behavior, 17 cases were classified as favorable, 26 as unfavorable, and 14 as borderline. Among the favorable and unfavorable groups, only the presence of mitoses, mitoses near the base, and an inflammatory reaction were statistically significantly associated with unfavorable behavior. There were no statistical significant differences among groups for ulceration, tumor thickness, and symmetry.

**Conclusions:** This study confirmed the difficulty in making a definitive diagnosis in these challenging cases and matching the histopathologic diagnosis with behavior. The authors' preliminary conclusion is that these lesions represent a biological group different from conventional melanoma and benign melanocytic nevi. In fact, this study shows that there may be a group of melanomas even with lymph node deposits that rarely give rise to distant metastases.

**Reviewer's Comments:** The authors of this paper represent a group of dermatopathologists and pathologists with extensive experience in melanocytic lesions. Their findings underscore the significant difficulties that exist with the more unusual yet commonplace cases, melanocytic lesions. Interestingly, this group may be biologically different than conventional melanoma or benign nevi, and there will assuredly be further studies, especially molecular, on these lesions. (Reviewer-William A. Kanner, MD).

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Keywords: Melanoma

Print Tag: Refer to original journal article
The relapse rate is greater in TTP patients with an ADAMTS13 activity of ≤10%.

**Background:** For patients with thrombotic thrombocytopenic purpura (TTP), plasma exchange therapy (PEX) has dramatically improved survival (prior to PEX, only 10% of patients survived). Although ADAMTS13 deficiency is part of the pathogenesis of and specific for TTP, the patient population is heterogeneous. The level of activity that is clinically significant and defines severe disease has not been established. In idiopathic TTP, patients have severe ADAMTS13 deficiency without other causes. These patients have increased survival compared to those diagnosed with secondary TTP. Secondary TTP patients rarely have severe ADAMTS13 deficiency, have other conditions that may cause thrombotic microangiopathy, and have poor survival.

**Objective:** To report the experience of the Oklahoma TTP Registry with regard to clinical outcomes and relapse in patients with TTP.

**Methods:** The Oklahoma Blood Institute is the sole provider of PEX throughout most of the state. Consecutive patients have been enrolled in this registry since 1989, and data have been collected prospectively. ADAMTS13 activity and inhibitor measurements were performed.

**Results:** 376 patients clinically diagnosed and treated with PEX who were followed up over 20 years were studied. In all patients, survival rates did not change over this period. Patients with idiopathic TTP had increased survival (80%) compared to all TTP patients. Interestingly, patients with ADAMTS13 activity <10% did not have a significantly different survival (78%) compared to patients with an activity of >10% (68%). However, those with an activity of ≤10% and an inhibitor titer of ≥2 Bethesda units/mL had a lower survival rate. The relapse rate was greater among patients with an ADAMTS13 activity ≤10%, with an estimated risk of relapse of 41% at 7.5 years. However, ADAMTS13 deficiency during remission was not clearly related to subsequent relapse.

**Conclusions:** Although there is greater understanding of and better treatment for TTP, mortality and morbidity remain substantial. There is still much heterogeneity among these patients, and the dichotomous classification of idiopathic and secondary TTP is not completely accurate. In this study, among those diagnosed with idiopathic TTP, only half the patients had an ADAMTS13 activity of ≤10%. Most initial relapses for patients with activity ≤10% occurred within the first year after remission, and the rate of relapse decreased with time. The authors do note that analysis of the relapse rate may be confounded with the increasing use of rituximab in these patients.

**Reviewer’s Comments:** Mortality has not changed significantly since the introduction of PEX, and this therapy is not without its complications. However, there have been significant improvements in the understanding of and diagnosis of TTP. With ADAMTS13 measurements becoming more common, this may lead to more insight into classifying these patients. (Reviewer-William A. Kanner, MD).

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Keywords: Thrombotic Thrombocytopenic Purpura, ADAMTS13

Print Tag: Refer to original journal article
P16 expression can be seen in benign cystic squamous lesions of the head and neck.

Background: Patients with squamous cell carcinoma of the head and neck frequently present with cervical lymph node metastasis as the initial presentation. In fact, the primary tumor site in these cases is often occult, and the initial clinical impression is of a benign process. Metastatic squamous cell carcinomas in this region also frequently undergo cystic degeneration, resulting in confusion with other benign cystic lesions of the neck. In a significant number of these cystic squamous cell carcinomas, tumorigenesis is related to HPV infection, resulting in p16 up-regulation. Therefore, various authors have suggested p16 immunostaining may be helpful in distinguishing benign from malignant lesions.

Objective: To evaluate the distribution of p16 staining in a variety of benign and malignant squamous cysts of the neck.

Methods: Surgical and fine-needle aspiration (FNA) cases of non–HIV-related lymphoepithelial cysts of the neck and parotid gland were selected. An additional group of FNAs interpreted as metastatic squamous cell carcinoma in the neck were included. A representative formalin-fixed tissue block or cell block from each case was immunohistochemically stained with an antibody against p16. Strong nuclear and cytoplasmic staining for p16 was considered positive. In addition, in situ hybridization for HPV16 was performed on each case; the presence of punctuate hybridization signals located within nuclei defined HPV positivity.

Results: 49 lymphoepithelial cysts (37 surgical specimens, 12 FNA) and 16 metastatic squamous cell carcinomas (all FNA) were examined. Patchy strong p16 staining was present in 21 (42%) of lymphoepithelial cysts. Strong p16 staining was present in 3 (19%) metastatic squamous cell carcinomas. HPV in situ hybridization was positive in all 3 metastatic squamous cell carcinomas that were positive for p16 and were negative in the remaining metastatic squamous cell carcinomas and all lymphoepithelial cysts.

Conclusions: The presence of strong p16 staining in a cystic squamous-lined cyst of the neck is not useful in separating benign from malignant lesions. P16 gene expression is not always linked to high-risk HPV infection.

Reviewer's Comments: While it is a good surrogate marker for HPV infection in morphologically diagnostic squamous cell carcinomas, p16 can be up-regulated in normal epithelium and other processes. A recent case from my practice serves a point of caution: a malignant tumor of the nasal cavity with squamous differentiation was strongly p16 positive. However, her clinical history was significant for a long-standing history of basal cell carcinoma in this region. Basal cell carcinomas can be p16 positive, and HPV in situ hybridization was negative in this case. (Reviewer-Deborah J. Chute, MD).

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Keywords: Neck, Squamous Cell Carcinomas, HPV16, Lymphoepithelial Cysts

Print Tag: Refer to original journal article
Background: Mucosal melanomas of the sinonasal tract are infrequent, and have different clinical and histopathologic features than cutaneous melanomas. Most studies are retrospective due to the rarity of these lesions; many extend several decades to gather enough cases for statistical significance. However, any advantages of recent advances in diagnosis and treatment are likely obscured by older data.

Objective: In this study from M.D. Anderson, the authors reviewed the clinical and pathologic characteristics of sinonasal mucosal melanoma and outcomes over the last 15 years.

Methods: Consecutive patients treated for sinonasal mucosal melanoma with histologic confirmation available for review were included in the study. Clinical data and treatment information were retrieved from the medical record system. Pathology specimens were reviewed by one experienced head and neck pathologist for features related to outcome. All patients were staged retrospectively using the 2003 American Joint Committee on Cancer (AJCC) staging system for sinonasal tumors. Overall survival was calculated at 5 years.

Results: 58 patients with a mean age of 63 years were diagnosed with sinonasal melanoma during the study period. The mean follow-up was 34 months. Melanomas arising from the nasal septum had a higher overall survival rate than those from other sites (50% vs 20%), although this with correlated with lower stage of disease in the septal group. According to the AJCC staging system, 27% of patients were classified as T1, 33% as T2, 21% as T3, and 19% as T4. Improved overall survival was statistically correlated with lower tumor stage. Patients with negative margins had better overall survival (44% vs 25%), although this difference was not statistically significant. Histopathologic features associated with worse outcome included the presence of >10 mitotic figures per HPF, increased tumor pigmentation, and the presence of a pseudopapillary tumor architecture. Surgical resection was the treatment of choice; postoperative radiation therapy did not improve overall survival but did improve locoregional recurrence.

Conclusions: The AJCC staging system is an effective outcome predictor and should be the staging system of choice. Histologic features associated with a worse outcome (increased mitotic activity, pseudopapillary architecture, and increased pigmentation) should be reported when present.

Reviewer's Comments: The failure of margin status to significantly predict outcome is interesting, as surgical resection is the treatment of choice. This may be due in part to the difficulty in accurately assessing the margin status in many of these patients. Both the complex anatomy and the subtle appearance of mucosal melanoma can make this challenging even for experienced pathologists. (Reviewer-Deborah J. Chute, MD).
What Is Spectral Cytopathology?

Cytopathology by Optical Methods: Spectral Cytopathology of the Oral Mucosa.

Papamarkakis K, Bird B, et al:

Lab Invest 2010; 90 (April): 589-598

Spectral cytopathology may be able to detect disease within cells before any morphologic abnormalities are visible using bright field microscopy.

**Background:** Spectral cytopathology (SCP) uses the principles of vibrational spectroscopy to measure differing infrared (IR) radiation absorption patterns among various cell types. These differing absorption patterns reflect differences in the underlying biochemical makeup of the cell, such as the amount of keratin intermediate filament. These results may be used to compile a "spectral fingerprint" for normal epithelial cells as well as for cells showing reactive, premalignant, and malignant change.

**Objectives:** To describe spectral cytopathology and its potential application in the evaluation of normal and abnormal epithelial cells of the oral mucosa.

**Methods:** Normal oral cytology specimens were obtained from laboratory volunteers such that 5 regions of the mouth were sampled: the tongue, hard palate, cheeks, gums, and floor of mouth. For "abnormal" cells, residual specimens from clinical samples that had undergone prior testing and diagnosis were used. Spectral data acquisition consisted of imaging a 4 x 4 mm spot on a microscopic slide that contained the exfoliated cells. These cells were scanned through the focus of a beam of IR light. Raw data were analyzed by calculating the cellular spectrum for each contiguous area within a cell. The cells were then Pap-stained and digitally imaged. Each cell image was stored with its associated spectral data.

**Results:** SCP was able to reproducibly classify normal oral squamous cells according to the anatomic site. Using clinical samples, SCP was able to distinguish among cells showing infection (herpes simplex virus), reactive atypia, and squamous cell carcinoma, among others.

**Conclusions:** SCP is able to detect disease within cells of the oral cavity before any morphologic abnormalities are visible using bright field microscopy. Thus, SCP shows promise as a potentially important ancillary tool in the practice of cytopathology.

**Reviewer's Comments:** This technology (referred to as "automatic cytopathology" in the paper) and its potential application are worth knowing about. In theory, SCP may be able to detect the underlying biochemical changes within a cell before the morphologic alterations are visible using bright field microscopy. Although the "unsupervised" nature of the testing and spectral pattern acquisition does not require subjective interpretation during each respective stage of testing, the end results must eventually be "interpreted." (Reviewer-T. David Bourne, MD).

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Keywords: Cytopathology, Image Analysis

Print Tag: Refer to original journal article
As measured using image cytometry, diploidy is more commonly associated with the reticular subtype of oral lichen planus, whereas aneuploidy is more commonly associated with the atrophic-erosive subtype.

**Background:** Oral lichen planus (OLP), which has been classified into reticular oral lichen planus (ROLP) and atrophic-erosive oral lichen planus (AEOLP), has been defined by the World Health Organization as a precancerous lesion. Debate about the actual risk of malignant progression, however, is still ongoing. The use of ploidy analysis to classify and diagnose various reactive and neoplastic lesions has been documented. One way to measure the DNA content of a cell is through image cytometry, a method that involves the densitometric measurement of DNA content within a cell nucleus with comparison to the DNA content of cells with known quantities of nucleic acid.

**Objective:** To perform DNA ploidy analysis using image cytometry in cases of OLP in an attempt to better define the malignant potential of this disease and its subtypes.

**Methods:** 40 cases from patients with the clinical and biopsy findings of OLP were studied (20 cases of ROLP and 20 cases of AEOLP). A single slide from each case was prepared for image cytometric analysis, which consisted of automated capture of 40 to 80 consecutive fields to include the basal, parabasal, and spinous layers of the epithelium. Sixty nuclei from each case were randomly imaged and compiled into an image karyogram. To provide a diploid (2n) reference, the nuclei of 20 lymphocytes were also studied. Karyometric measurements included some of the following parameters: area, maximum diameter, circumference, mean density, and optical density. Ploidy determinations were calculated based on the integrated optical density of the nuclei.

**Results:** The most common degree of DNA ploidy among OLP cases was diploidy. Among cases of ROLP, there were 18 diploid samples and 2 aneuploid samples. Among AEOLP cases, there were 10 diploid samples, 1 tetraploid sample, and 9 aneuploid samples.

**Conclusions:** Diploidy is more commonly associated with the ROLP subtype of OLP, while aneuploidy is more commonly associated with the AEOLP subtype ($P = 0.021$).

**Reviewer’s Comments:** The findings in this study support prior observations linking the AEOLP subtype with the development of cancer. However, the conclusions would certainly be strengthened by following a larger study cohort over a long period. (Reviewer-T. David Bourne, MD).

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Keywords: Oral Lichen Planus, Image Cytometry, Ploidy

Print Tag: Refer to original journal article
Angiomatoid fibrous histiocytomas frequently have \textit{EWRS1} and may rarely have \textit{FUS} rearrangements by FISH.

**Background:** Angiomatoid fibrous histiocytoma (AFH) is a rare mesenchymal neoplasm typically found in children and young adults, particularly in the extremities. The lesion is of intermediate malignancy, as some cases have recurred locally and rare cases have metastasized. The histologic appearance of AFH is fairly characteristic, including bland histiocytoid cells forming pseudovascular spaces surrounded by a lymphoid cuff and fibrous capsule that imparts the appearance of a lymph node metastasis. The classic features are not always present or appreciated, and there is no specific immunohistochemical stain to suggest or confirm the diagnosis. Several reports have noted molecular abnormalities in AFH, including gene fusions involving \textit{EWSR1} and \textit{FUS}, which are commonly evaluated by FISH in larger medical centers evaluating common soft-tissue tumors.

**Objective:** To evaluate a larger series of AFH for rearrangements in \textit{EWRS1} and \textit{FUS} by FISH for possible adjunctive diagnostic testing.

**Methods:** 4 initial cases of \textit{EWRS1} rearrangements were identified, and then subsequent archived cases were collected for 18 cases confirmed by 2 soft-tissue pathologists. Immunohistochemical stains were reviewed or performed. FISH for \textit{EWRS1} and \textit{FUS} were performed.

**Results:** Desmin was positive in all but one case, but some cases were focal. CD68, CD99, and EMA were each positive in at least half of the tumors when performed, and SMA was positive in about one-third. FISH detected rearrangements in \textit{EWRS1} in 76\% of 17 cases (one was technically unsatisfactory), and a rearrangement in \textit{FUS} was not detected in any case.

**Conclusions:** AFH frequently has rearrangements in \textit{EWRS1}, but 24\% of cases were negative in this study. \textit{FUS} rearrangements were not identified but have been noted in the literature. The immunohistochemical and FISH results may overlap with Ewings/PNET, which should be excluded by histology and clinical presentation.

**Reviewer's Comments:** AFH has a characteristic clinical presentation and histology, but difficult cases may be aided by FISH results since many have \textit{EWRS1} rearrangements. (Reviewer-Mary T. Galgano, MD).
CNS DLBCL Subtypes Lack Prognostic Significance

Most Primary Central Nervous System Diffuse Large B-Cell Lymphomas Occurring in Immunocompetent Individuals Belong to the Nongerminal Center Subtype: A Retrospective Analysis of 31 Cases.

Hattab EM, Martin SE, et al:

Mod Pathol 2010; 23 (February): 235-243

DLBCL is the most common primary CNS lymphoma and is increasing even in immunocompetent patients. Most of these cases are of nongerminmal center origin, but the prognosis is no different from those with germinal center origin.

**Background:** Nodal and extranodal diffuse large B-cell lymphomas (DLBCL) have been determined to have prognostically significant subtypes by gene expression profiles. These correlate with germinal center phenotype versus nongerminmal center phenotype, which can be characterized by a limited immunohistochemistry (IHC) panel, including CD10, Bcl-6, and MUM1. CD10 with or without Bcl-6 positivity indicates the germinal center phenotype. If both CD10 and Bcl-6 are negative, the MUM1 classifies the lymphoma as nongerminmal center phenotype if positive, and germinal center phenotype if negative. In nodal DLBCL, those with germinal center phenotype have a better prognosis than those with nongerminmal center phenotype.

**Objective:** To apply the immunohistochemical panel to primary central nervous system (CNS) DLBCL for characterization and prognostic significance.

**Methods:** 31 cases of CNS DLBCL were identified from the archived files with sufficient material available for further studies. These were subjected to IHC studies and scored as positive if >30% of tumor cells reacted. The tumors were subclassified as described above and correlated with clinical outcome of the patient. FISH was performed if tissue was available.

**Results:** CNS DLBCL occurred in patients from age 13 to 81 years (mean age, 62 years); 94% were Caucasian, and 65% were women. None were known to be HIV-positive, although one was a liver transplant patient. IHC results stratified 84% as nongerminmal center subtype, and these cases had a Ki-67 proliferation rate of 70% to 90%. The other 5 cases were germinal center subtype and had a similar range of Ki-67 proliferation. FISH results were available in 21 cases, and 38% had abnormalities of MYC/IGH and/or IGH/BCL2. With a mean follow-up of 54.3 months, there was no survival difference between the IHC-defined subtypes using Kaplan-Meier analysis.

**Conclusions:** While most cases of primary CNS DLBCL are of the nongerminmal center subtype, this does not appear to confer a worse prognosis compared to the germinal center subtype, as has been noted in nodal DLBCL.

**Reviewer's Comments:** Although tumors with “Burkitt-like” morphology were excluded, the authors note high proliferation rates and frequent abnormalities by FISH in the entire cohort of primary CNS DLBCL. (Reviewer-Mary T. Galgano, MD).

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Keywords: Diffuse Large B-Cell Lymphoma

Print Tag: Refer to original journal article
Breast Cancer Metastases -- Meta-Analysis

Breast Cancer Prognosis and Occult Lymph Node Metastases, Isolated Tumor Cells, and Micrometastases.

de Boer M, van Dijck JAAM, et al:

J Natl Cancer Inst 2010; 102 (March 17): 410-425

Data regarding the outcomes for patients with micrometastases or occult metastases seen in sentinel nodes remain mixed.

**Background:** Axillary lymph node status is the most predictive factor of outcome with breast cancer, and greater numbers of involved nodes are associated with worse outcomes. For >50 years, it has been known that serial sectioning of axillary lymph nodes helps identify metastases that are not seen with routine sectioning. Some have termed these "occult" metastases. Serial sectioning is more commonly performed with sentinel lymph nodes, however, and the question remains as to whether the identification of small metastases through these procedures provides prognostically significant information. Distinction has also been made between metastases <0.2 mm in size (isolated tumor cells) and those between 0.2 and 2 mm in size (micrometastases). Patients with micrometastases in sentinel nodes are at 20% risk for having other involved nodes, and those with isolated tumor cells are at 12% risk for having other involved nodes.

**Objective:** This manuscript compared survival outcomes for patients with "occult" metastases, isolated tumor cell metastases, and micrometastases.

**Methods:** A literature review was conducted to include all papers that studied the relationships between "occult" metastases, isolated tumor cell metastases, micrometastases, and survival. Papers were included if results were compared to lymph node-negative patients. Data were compared regarding the degree of sectioning. "Occult" metastases were those identified with more extensive sampling and sometimes immunohistochemical staining.

**Results:** 46 articles were selected, which included 58 studies and nearly 300,000 patients. Of studies investigating micrometastases with axillary dissections, 7 of 9 showed worse disease-specific survival for patients with micrometastases. Eight of 8 studies showed worse overall 10-year survival for patients with micrometastases. Studies regarding "occult" metastases were very heterogeneous in their sampling methods. Pooled data showed increased risk for recurrence and death for patients found to have occult metastases. In these studies, micrometastases were associated with reduced disease-free or overall survival rates. All but 1 of 5 studies showed isolated tumor cells to be associated with reduced disease-free and overall survival. By multivariate analysis, however, occult metastases were associated with disease-specific survival in only a minority of studies. Five of 5 studies of sentinel lymph node biopsy papers showed no reduced recurrence-free survival for patients with micrometastases.

**Conclusions:** Metastases <2 mm detected by routine sectioning for patients with breast cancer who undergo axillary dissection are associated with poorer outcomes. Data are more mixed for disease found with sentinel node biopsy and additional histologic sampling.

**Reviewer's Comments:** This meta-analysis is a good read for surgical pathologists who examine breast cancer resections as it carefully describes much of the literature regarding axillary lymph node metastases. It may assist them in their choices regarding the sampling of sentinel lymph nodes. (Reviewer-Erward B. Stelow, MD).

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Keywords: Micrometastases, Occult Metastases, Isolated Tumor Cells, Survival

Print Tag: Refer to original journal article
The degree of downstaging was the only measurement of response that predicted disease-free and overall survival in this study by multivariate analysis.

**Background:** Preoperative radiochemotherapy is considered standard treatment for patients with locally advanced rectal adenocarcinomas. Radiotherapy was originally shown to decrease recurrence rates with rectal adenocarcinoma compared to optimal surgical resection. In addition, chemotherapy paired with radiotherapy decreased the risk for recurrence by half for these tumors compared to radiotherapy alone. Preoperative therapy typically gives rise to pathologic changes, including tumor cell death and downstaging.

**Objective:** To investigate multiple pathologic assessments of tumor response for neoadjuvantly treated rectal carcinoma and their associations with patient survival.

**Methods:** Over a 10-year period, all patients with locally advanced (pT3 or T4 or N+) rectal adenocarcinomas who underwent surgical excision were initially included. Patients with metastatic disease were excluded. Preoperative treatment included radiation therapy of 45 Gy over 5 weeks and continuous 5-fluorouracil therapy. Tumors were pathologically assessed for complete response, good response (downstaged to ypT0-2, N0), or bad response. Response was also graded in two 5-tiered systems and was compared to survival.

**Results:** The median patient age was 65 years, and most patients had undergone low anterior resection. Most patients received preoperative chemotherapy and radiotherapy. The average tumor size was 2.7 cm. Nearly 90% of patients had what was considered to be an R0 resection. The disease-free and overall survival rates at 5 years were 64% and 75%, respectively. Local and distant recurrences were diagnosed in 7 and 65 patients, respectively. The mean time to recurrence was 17 months. All measurements of tumor response (except for complete response) were associated with disease-free and overall response by univariate analysis. By multivariate analysis, only response as determined by degree of downstaging was associated with both disease-free and overall survival. Patients considered to be complete or good responders had a 5-year disease-free survival rate of 85%, whereas bad responders had a 5-year disease-free survival rate of 55%. Other factors associated with survival included age, positive circumferential margin, vascular invasion, and post-therapy positive lymph nodes.

**Conclusions:** With locally advanced rectal adenocarcinoma, only the degree of downstaging independently predicts disease-free and overall survival.

**Reviewer's Comments:** This study is akin to many that pathologists must be familiar with as more and more specimens received in the surgical pathology laboratory have already undergone chemotherapy or radiotherapy. Hopefully, more studies like this will eventually lead to simplified tumor reporting guidelines. (Reviewer—Edward B. Stelow, MD).

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**Keywords:** Adenocarcinoma, Chemotherapy, Radiation, Response, Survival

**Print Tag:** Refer to original journal article