The distribution of FOXP3-positive T cells is an important predictor of overall survival and risk of transformation in patients with follicular lymphoma.

**Background:** There is significant clinical heterogeneity in follicular lymphoma (FL), and although indolent, FL can transform into an aggressive lymphoma. Currently, there are no consistent biological markers identified that predict the risk of transformation and patient survival. Recent research has focused on the tumor microenvironment (ie, non-neoplastic immune cells). Regulatory T cells (Tregs) are known to suppress effector T cells and prevent reactivity to self-antigens. Thus Tregs may recognize specific tumor antigens, differentiate, and suppress naive and CD4+ Th1 antitumor effector cells. When Tregs are depleted, tumor surveillance is increased. FOXP3 is a transcriptional factor and the key control gene in Treg development. Intratumoral FOXP3+ T cells have been shown to migrate or be induced by malignant B cells and then have the ability to suppress the function of other infiltrating T cells.

**Objective:** To analyze the role of Tregs in FL in terms of numbers and distribution in the tumor microenvironment with a patient cohort that has been uniformly treated and has long-term follow-up.

**Methods:** In this retrospective review, consecutive patients were seen and treated with multiagent chemotherapy (BP-VACOP) and radiation during a 5-year period. The International Prognostic Index (IPI) score was calculated. All pathology was reviewed and graded according to the World Health Organization 2008 criteria. A tissue microarray was constructed, and the prepared slides were stained with H&E and antibodies to CD4, CD8, CD20, CD21, CD25, and FOXP3. FOXP3- and CD25-positive cells were counted, and the pattern of distribution was characterized as either follicular or diffuse (ie, no relationship to the neoplastic follicles). Statistics and survival analysis was also completed.

**Results:** There were 102 patients in this study, with a median follow-up of 17 years. By histology, there were 80 grade 1 FLs, 16 grade 2 FLs, and 6 grade 3A FLs. Thirty-eight cases had a follicular/perifollicular pattern with FOXP3+ cells, and 64 cases had a diffuse pattern. Statistically, the IPI and the FOXP3+ pattern were independent predictors of overall survival, while in a multivariate Cox model analysis, only the FOXP3+ pattern showed significance in determining the risk of transformation. In both instances, a follicular pattern of FOXP3+ staining was associated with decreased overall survival and increased risk of transformation than with the diffuse pattern. Finally, the cell content (quantitative analysis of Tregs) did not impact survival.

**Conclusions:** The immunoarchitectural pattern of Tregs within the lymph nodes of patients with FL predicts survival and risk of transformation. Importantly, these patients were uniformly treated.

**Reviewer's Comments:** There is active investigation into what the tumor microenvironment may tell us about how the tumor will behave and how it interacts with our immune system. This article uses that information and demonstrates a correlation with overall survival and risk of transformation in patients with follicular lymphoma.

(Reviewer-William A. Kanner, MD)

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Keywords: FOXP3, Follicular Lymphoma, Regulatory T Cells

Print Tag: Refer to original journal article
Podoplanin Is Still a Useful Marker for Lymphatic Space Invasion

Podoplanin Expression in Basal and Myoepithelial Cells: Utility and Potential Pitfalls.

Kanner WA, Galgano MT, Atkins KA:


D2-40 consistently highlights lymphatics, and staining of myoepithelial cells is weaker and more focal than seen in lymphatic spaces.

**Background:** Podoplanin is a transmembrane glycoprotein that was first described in lymphatic epithelium. The antibody to podoplanin, D2-40, is well known for its ability to highlight lymphatics, a particularly useful tool when assessing for angiolymphatic invasion. However, D2-40 has recently been reported to stain other normal tissues, including myoepithelial and basal cells. This is particularly concerning in patients with breast carcinoma with a prominent ductal carcinoma in situ (DCIS) component where myoepithelial cells could be mistaken for lymphatic space invasion.

**Objective:** To evaluate the utility and pitfalls of using D2-40 to detect lymphatic invasion in tumors from organs with a prominent myoepithelial or basal cell component.

**Methods:** Archival formalin-fixed paraffin-embedded tissue blocks from tumors with the following profiles were retrieved: 18 breast carcinomas; 10 prostatic adenocarcinomas; 7 cutaneous basal cell carcinomas; 7 cervical carcinomas; and 7 salivary gland tumors (3 salivary duct, 1 pleomorphic adenoma, 1 carcinoma expleomorphic adenoma, 1 acinic cell, and 1 mucoepidermoid carcinoma). The breast cases were divided into invasive micropapillary carcinomas (10 cases) and invasive ductal carcinomas with a prominent DCIS component (8 cases). All tissue blocks had a combination of tumor and normal epithelium. All cases were stained with D2-40, and breast cases were also stained with p63 for myoepithelial confirmation. Cytoplasmic D2-40 staining was assessed in lymphatics, myoepithelial cells, and tumor cells in each case.

**Results:** In all cases, lymphatic spaces showed strong and diffuse positivity with D2-40. Normal myoepithelial/basal cells were positive with D2-40 in all cases of breast, salivary gland, skin, and cervix tissues, although the staining was typically patchy and less intense than seen in lymphatic spaces. P63 staining in breast cases confirmed D2-40 localization in myoepithelial cells. Tumor cells were focally positive in some salivary gland tumors (pleomorphic adenomas and mucoepidermoid carcinoma), 1 case of basal cell carcinoma, and 2 cases of cervical carcinoma. The neoplastic cells were completely negative in all breast and prostate cancers. The retraction artifact seen around tumor nests in invasive micropapillary carcinoma of the breast was negative in all cases. Myoepithelial cells surrounding DCIS were positive for D2-40, but staining was less intense and focal than in normal ducts.

**Conclusions:** D2-40 consistently highlights lymphatics. Although it frequently marks myoepithelial/basal cells, the staining is weaker and focal than seen with lymphatics. Using D2-40 in conjunction with a myoepithelial cell marker, like p63, can help prevent misinterpretation of in situ carcinoma as lymphovascular space invasion.

**Reviewer's Comments:** D2-40 is a very helpful marker of angiolymphatic invasion, particularly when combined with CD31. This study demonstrates that podoplanin expression is useful for identifying lymphatic invasion in breast and prostate cancers, but its utility in other organ sites will require additional studies.

(Reviewer-Deborah J. Chute, MD).

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Keywords: Podoplanin, D2-40, Invasive Micropapillary Carcinoma, Prostatic Adenocarcinoma, Invasive Ductal Carcinoma, Myoepithelial, Salivary Gland, Cervix, Skin, Basal Cell Carcinoma

Print Tag: Refer to original journal article
**Background:** Pancreatic ductal adenocarcinoma (PDAC) remains one of the leading causes of cancer mortality, in large part due to difficulties in early detection. Precursors of PDAC are thought to include intraductal papillary mucinous neoplasms, mucinous cystic neoplasms, and pancreatic intraepithelial neoplasia (PanIN). PanIN lesions have recently been shown to harbor activating point mutations in the KRAS gene, and mice with mutated KRAS have been found to have pancreatic lesions that appear identical to those seen in humans. MicroRNAs (miRs) are noncoding RNAs that negatively regulate gene expression at the posttranscriptional level, and microRNA alterations have been linked to the initiation and progression of PDAC, among other cancers. Thus, their detection may be useful as a tool in early tumor diagnosis. Since resection of early-stage PDAC is associated with 5-year survival rates approaching 40%, having an accurate and reliable biomarker for early PDAC detection could significantly improve outcomes.

**Objectives:** To determine whether abnormal microRNA production, which occurs in PDAC, also occurs in various PanIN lesions.

**Methods:** Laser-capture microdissection was used to obtain cells from normal pancreas and PanIN lesions in both human pancreatic tissues as well as pancreatic tissue from a conditional KRAS mouse model. Quantitative real-time PCR (qRT-PCR) was used to determine the amount of microRNA production in each of the samples. In situ hybridization (ISH) assays were also performed for microRNA detection.

**Results:** An initial panel of candidate microRNAs known to be associated with cancer was assessed in 7 cases of PDAC in tandem with normal control tissues. The panel included miR-221, miR-222, let-7a, miR-29c, miR-200, and miR-205, all of which are known to be involved in various cell regulatory functions such as cell proliferation and tumor angiogenesis. In mouse tissues, qRT-PCR studies showed that miR-21, miR-205, and miR-200 production paralleled the progression of PDAC. In human samples, the production of miR-21, miR-221, miR-222, miR-200, and miR-205 increased with increasing PanIN grade. miR-21 showed the highest relative concentrations in both mouse and human samples. ISH analysis confirmed the presence of miR-21 in human PanIN lesions. Cell culture studies of PDAC cell lines showed that miR-21 production appeared to be regulated by KRAS and epidermal growth factor receptor.

**Conclusions:** As is seen in PDAC, aberrant microRNA expression (such as miR-21) does occur in PanIN lesions in both human and mouse tissue. Given its relatively high concentration among PanIN lesions, miR-21 may hold promise as an early detection biomarker of PDAC.

**Reviewer’s Comments:** This well-designed study confirms the rationale for investigating the potential role of miR-21 as a biomarker for the early detection of PDAC. Other forms of nucleic acid testing, such as cell-free DNA analysis, may also be useful in this setting. (Reviewer-T. David Bourne, MD).

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**Keywords:** Molecular Testing, Pancreatic Ductal Adenocarcinoma

**Print Tag:** Refer to original journal article
Screening and imaging have led to the overdiagnosis of malignancy.

**Background:** Over the years, the diagnosis of "cancer" has increasingly come to mean less. At one time, it could simply be said to mean a neoplasm that, if left untreated, would generally result in the death of the patient. This had been mostly true when cancers were diagnosed that only came to attention because of clinical reasons. As improved technology has led to the detection of many cancers that are not associated with clinical manifestations, the diagnosis of cancer has come to mean less prognostically.

**Objective:** This article reviews the overdiagnosis of cancer. The authors describe overdiagnosis here as a condition that, if left untreated, would not lead to symptoms or death.

**Results:** Overdiagnosis with cancer happens when the cancers will either not progress (or even regress, such as with neuroblastoma) or when the patient dies of other causes prior to the cancer causing symptoms. Unfortunately, clinicians cannot know if a case represents an overdiagnosis unless the patient is not treated and dies from another cause prior to developing symptoms from their disease. From autopsy studies, it has been known that there is a considerable subclinical cancer disease reservoir. For example, from autopsy studies of men who died of other diseases, it is estimated that 30% to 70% of men >60 years of age have subclinical prostate cancer, that up to 36% to 100% of adults have papillary thyroid carcinomas, and that 7% to 39% of adult women have breast cancer. Disease reservoir by itself does not lead to overdetection. This generally happens because of screening, motivated by the fact that cancers detected at a lower stage tend to do better when treated. Mammography and prostate-specific antigen (PSA) testing are examples of screening tests that have led to overdiagnosis. Increased diagnostic imaging used for nonscreening purposes also frequently leads to detection of nonsymptomatic cancers. There is a great deal of evidence supporting the claims of overdiagnosis. Screening populations are diagnosed with more cancers than control populations, even with extremely prolonged follow-up periods. Examples include breast, prostate, and even lung cancer. The incidence rates of a number of cancers have drastically increased over the past 30 years, while little has changed with the mortality rates for those cancers. Examples here include breast, prostate, thyroid, kidney, and skin cancers. For example, the rate of diagnosis of thyroid cancer has more than doubled over the past 30 years, while mortality has not changed.

**Conclusions:** The overdiagnosis of cancer has made choices about screening and treatment more complex. Patients must be informed about the tradeoff for screening and treatment of their disease.

**Reviewer's Comments:** This article highlights problems with a purely histologic definition of cancer. Interestingly, in spite of this difficulty, diagnostic thresholds for some malignancies continue to decline. (Reviewer-Edward B. Stelow, MD).

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Keywords: Cancer, Screening, Overdiagnosis, Thyroid, Breast, Prostate

Print Tag: Refer to original journal article
Cyclin D1 is increased in reactive and metaplastic endocervical glands, but negative/focal in AIS and invasive adenocarcinomas.

Background: Endocervical adenocarcinoma in situ (AIS) and invasive adenocarcinoma are increasing in incidence, but can be more difficult to screen, follow, and treat than cervical squamous dysplasia. The histologic diagnosis of AIS can be complicated by reactive and metaplastic changes, small biopsy samples, and procedural and technical artifacts. However, both underdiagnosing and overdiagnosing AIS can have profound clinical implications. Nearly all AIS are HPV related and have associated alterations of the RB-p16-cyclin D1 pathway, thus p16 overexpression is a relatively potent marker for AIS. However, not all small laboratories have access to p16, and it is noted to be positive in some metaplastic epithelium of the cervix, such as tubal metaplasia, which is a frequent mimic of glandular dysplasia.

Objective: To determine whether cyclin D1 has diagnostic utility in endocervical AIS or invasive adenocarcinoma.

Methods: 64 cervical excisions were collected that included normal, neoplastic, and reactive/metaplastic endocervical lesions. These were stained for p16, ki-67, and cyclin D1. Specific patterns of staining were noted within lesions and assigned a quantitative score.

Results: Normal appearing endocervical cells generally expressed nuclear cyclin D1, but in a focal distribution, and few were negative. Reactive and metaplastic endocervical cells, including tubo-endometrioid cells, had increased expression of cyclin D1. Most of the lesions with tubo-endometrioid metaplasia had approximately 50% cells staining. The majority of these also stained at least focally for p16 and ki-67. Three cases of microglandular hyperplasia were diffusely positive for cyclin D1, but mesonephric duct remnants were very focally positive (<10% cells) for cyclin D1. All AIS had increased Ki-67, diffuse nuclear and cytoplasmic p16, and were usually negative for cyclin D1 (although few cases had <10% cells positive). Invasive adenocarcinomas were similar to AIS in that most were negative, but few had <10% cells positive for cyclin D1, usually at the advancing tumor margin.

Conclusions: Although there is overlap in staining profiles, cyclin D1 is generally positive in reactive and metaplastic cervical glandular epithelium, while generally negative in AIS and invasive adenocarcinoma. Since p16 and Ki-67 can be equivocal in reactive and metaplastic conditions, cyclin D1 may become a helpful addition to this panel.

Reviewer’s Comments: Before incorporating the use of cyclin D1 into this clinical use, pathologists should practice and get accustomed to the staining characteristics of their laboratory. However, the stain may be useful in that it seemed to best differentiate reactive/metaplastic lesions from neoplasia, where histology is most complicated and other stains may be equivocal. (Reviewer-Mary T. Galgano, MD).

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Keywords: Adenocarcinoma, Endocervical, Cyclin D1, Invasion, Immunohistochemistry

Print Tag: Refer to original journal article
The grading of tumor emboli in breast cancer has significant predictive value.

**Background:** Lymphatic space invasion (LSI) in breast cancer has long been considered to have prognostic significance. More recently, however, a grading system has been proposed for more accurately stratifying patients into risk categories.

**Objective:** To assess the utility of the LSI grading system to women with infiltrating ductal carcinoma (IDC) who received neoadjuvant therapy.

**Methods:** The pretreatment biopsy and surgical resection of consecutive breast cancer cases treated with neoadjuvant chemotherapy then surgery were reviewed for routine clinicopathologic features. The LSI was assessed by first identifying large vessel tumor emboli far from the stroma-invasive tumor margin. Some cases required D2-40 staining of lymphatic endothelial cells for confirmation of the tumor emboli. The mitotic and apoptotic figures within 1 high-powered field (HPF) of the tumor emboli were counted and graded as follows: grade 0, no lymph vessel invasion; grade 1, no mitotic or apoptotic figures or mitotic activity without apoptosis or apoptosis without mitotic activity; grade 2, 1 to 4 mitotic figures and at least 1 apoptotic figure, or at least 1 mitotic figure and 1 to 6 apoptotic figures; grade 3, >4 mitotic figures and >6 apoptotic figures. The HPF with the highest counts was recorded with other clinicopathologic data.

**Results:** The grade of tumor emboli in the biopsy and surgical specimen were both significantly associated with the number of nodal metastases. On multivariate analysis of traditional prognostic factors and p53 expression (using Allred scores), the tumor emboli grade on both biopsy and surgical specimen significantly increased the hazard rate for recurrence and tumor-related death, although the predictive power of the surgical specimen was superior to that of the biopsy. The prognostic value was significant for the group as a whole, those with nodal metastases and those without a nodal metastasis.

**Conclusions:** The grading of tumor emboli in women with IDC treated with neoadjuvant chemotherapy has independent predictive value for number of lymph node metastases and overall outcome.

**Reviewer's Comments:** The authors have previously reported similar results for women with IDC that did not undergo neoadjuvant chemotherapy, including risk stratification for overall outcome. (Reviewer-Stacey E. Mills, MD).

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Keywords: Lymphatic Space Invasion, Grading, Tumor Emboli

Print Tag: Refer to original journal article
Knowing the Morphological Characteristics of AFX

Background: Atypical fibroxanthoma (AFX) is a tumor that occurs on the sun-exposed skin in elderly patients. Histologically, it is composed of highly atypical cells and frequent mitoses admixed with spindled cells. The diagnosis is currently made by exclusion of other spindle cell tumors, such as spindle cell squamous cell carcinoma, sarcomatoid melanoma, and leiomyosarcoma. Although immunohistochemistry is helpful, especially in excluding melanoma, there are numerous pitfalls and dilemmas that arise when confronted with a lesion that may represent AFX.

Objective: The authors review their experience with AFX and give special emphasis on the different morphological variants, mimickers, and diagnostic pitfalls.

Methods: The cases consisted of referrals over a period of 5 years. Each case was studied for morphology and immunohistochemistry.

Results: There were a total of 66 cases, with the great majority (59 cases) representing male patients. The mean age was 77 years, and almost every case was from a sun-exposed region of the head (especially the scalp). On histologic examination, most cases had marked solar elastosis, and half had surface ulceration or were localized in the dermis. All cases had a mild intratumoral mononuclear inflammatory cell infiltrate. More cases demonstrated expansile rather than infiltrative growth, and vascular and perineural invasion were not present in the studied cases. Other changes noted in some cases included hemorrhagic/pseudo-angiomatous areas, granular cell change, keloid-like areas, myxoid degeneration, osteoclast-like giant cells, and clear cell change. Of the morphologic patterns, the most frequent was an admixture of spindle and epithelioid cells (histiocyte-like; 60.6%), followed by a predominantly spindle cell type and pure spindle non-pleomorphic cell type. Only a few cases consisted of a predominantly epithelioid cell type. By IHC, AFX cases were negative for S100 (although scattered dendritic cell staining was noted), CK-MNF116, AE1/AE3, and desmin. Focal staining was found with SMA, EMA, and CD31 in occasional cases. CD31 was notable for staining cells with more epithelioid morphology and tumor infiltrating macrophages.

Conclusions: There are several morphologic characteristics that one should be cognizant of in AFX. There are also various morphologic patterns, and one that may be easily overlooked is the spindle cell non-pleomorphic variant. However, the diagnosis of AFX is still a diagnosis of exclusion, and IHC plays a significant role in the differential diagnosis. S100 is negative in AFX; however, one may see positivity in intratumoral dendritic cells. Focal EMA and SMA reactivity can be seen, but should not be overinterpreted. A novel finding in this manuscript is the demonstration of CD31 positivity, possibly signifying histiocytic differentiation.

Reviewer's Comments: While much of the literature on AFX, as with many other tumors, focuses on immunohistochemical markers (CD10, CD99, procollagen 1), this practical paper focuses on the importance of morphology. (Reviewer-William A. Kanner, MD).

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Keywords: Atypical Fibroxanthoma, Morphology

Print Tag: Refer to original journal article
The presence of ASC-H cells, even in the setting of definitive LSIL, indicates a significant risk of CIN2 or worse on follow-up biopsy.

**Background:** The Bethesda System provides a universal classification system for cervical-vaginal Pap tests in order to better identify high-grade squamous intraepithelial lesions (HSIL). The category "atypical cells, cannot exclude HSIL" (ASC-H) is associated with a greater risk of cervical intraepithelial neoplasia (CIN) 2 or worse than the diagnosis of low-grade squamous intraepithelial lesion (LSIL). Rarely, cases fall into an indeterminate grade of dysplasia; in practice, these cases are often diagnosed as LSIL and ASC-H, or LSIL with a comment indicating features concerning for HSIL. Some authors have proposed a new category, "LSIL cannot rule out HSIL" (LSIL-H), but this remains highly controversial, with other authors favoring the use of ASC-H alone.

**Objective:** To compare the predictive value of Pap tests with an interpretation of ASC-H versus LSIL/ASC-H.

**Methods:** The archives at 1 institution were searched for women with follow-up cervical biopsies and a Pap test interpretation of LSIL/ASC-H or LSIL with a comment about possible HSIL. A control group of women with follow-up biopsies and a Pap test interpretation of ASC-H during the same period were also included. All Pap tests were examined using liquid-based cytology. Follow-up biopsies were limited to within 18 months of the index Pap test, and the most severe tissue diagnosis was considered the end diagnosis.

**Results:** 261 women with a Pap test of LSIL/ASC-H and 164 women with a Pap test of ASC-H met the inclusion criteria. The median patient age in the study and control groups was 28 and 31 years, respectively. On follow-up biopsy, 35% of women with a Pap test of LSIL/ASC-H and 38% of women with a Pap test of ASC-H had CIN2 or worse. This difference was not statistically significant. However, more women with a Pap test of LSIL/ASC-H had biopsy-proven CIN1 (45% vs 26%). If the biopsy results were divided into CIN2 and CIN3 groups, there was a larger proportion of CIN3 in the ASC-H group (21% vs 14%), while there was a larger proportion of CIN2 in the LSIL/ASC-H group (21% vs 17%).

**Conclusions:** The presence of ASC-H cells, even in the setting of definitive LSIL, indicates a significant risk of CIN2 or worse on follow-up biopsy. This suggests that cases of LSIL/ASC-H may need to be clinically followed in a manner similar to ASC-H.

**Reviewer’s Comments:** Although the predictive value for CIN2 or worse on follow-up was the same in both groups, the presence of a significantly increased number of CIN1 biopsies in the LSIL/ASC-H group suggests that these patients may represent a distinct subset of patients. In particular, the utility of HPV testing in these patients is questionable, as they would be expected to be positive in the majority of cases. (Reviewer-Deborah J. Chute, MD).

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Keywords: ASC-H, LSIL, HSIL, ASCCP Guidelines, ThinPrep, The Bethesda System

Print Tag: Refer to original journal article
Importance of Location, Morphologic Features in Pilocytic Astrocytoma

Impact of Morphology, MIB-1, p53 and MGMT on Outcome in Pilocytic Astrocytomas.

Certain histologic patterns in PA, such as a classic biphasic appearance, are more commonly observed in cerebellar versus noncerebellar tumors. Other morphologic features are associated with more favorable or less favorable outcomes.

**Background:** The most common glioma affecting pediatric patients is the pilocytic astrocytoma (PA), a low-grade tumor that usually carries a favorable prognosis. However, a minority of patients (even those with resectable tumors) have poor outcomes based on aggressive tumor behavior. Numerous studies have failed to demonstrate consistent results regarding the importance of morphologic features in prognosis. In addition, no panel of immunohistochemical stains has consistently shown prognostic value in helping to predict tumor behavior.

**Objectives:** To analyze a relatively large number of PAs to determine the impact of tumor location, morphology, MIB-1 staining, p53 expression, and \(O6\)-methylguanine-DNA methyltransferase (MGMT) promoter methylation status on patient outcome.

**Methods:** 147 cases, 118 of which had outcome data, were studied. Tumor locations included the cerebellum, brainstem, hypothalamus, thalamus, spinal cord, cortex, and basal ganglia. More than 90% of the midline tumors in the study were classified as subtotal resections, while >75% of the cerebellar and cortical tumors were classified as gross total resections. For purposes of the study, adverse outcomes included recurrence, progression, spread within the neuraxis, or death. H&E sections were reviewed for the presence of features such as cellularity, nuclear atypia, mitotic activity, oligodendroglioma-like areas, leptomeningeal dissemination, Rosenthal fibers, eosinophilic granular bodies, microcalcifications, and necrosis among others. Immunohistochemical staining for p53, MGMT, and MIB-1 was performed, as well as microdissection-based loss of heterozygosity studies or fluorescence in situ hybridization.

**Results:** 4 main histologic patterns of PA were observed: the classic biphasic pattern; a microcystic pattern; a dense piloid pattern; and a diffuse or "patternless" pattern. The classic biphasic pattern was most often seen in cerebellar tumors, while the microcystic pattern was observed most frequently among supratentorial, brainstem, and spinal cord tumors. While none of the histologic patterns showed a significant association with outcome, 3 of the individual histologic features did. Degenerative-type atypia was associated with a reduced risk of adverse outcome, oligodendroglioma-like areas within cerebellar tumors had a higher risk of adverse outcome, and leptomeningeal dissemination was associated with a lower risk of adverse outcome. The MIB-1 proliferation index, p53 expression, and MGMT expression were not associated with outcome.

**Conclusions:** Certain morphologic features are associated with adverse outcome in PA, but the utility of such features varies by tumor location. In contrast, immunostaining for MIB-1, p53, and MGMT showed no such prognostic value.

**Reviewer’s Comments:** Careful attention was paid to histologic findings in this study, and the results seem to warrant greater scrutiny of PAs for such features depending on the tumor location. It was interesting to note that cerebellar tumors with all 3 adverse risk factors had more than a 40% adverse outcome rate compared to a 7% adverse outcome rate among tumors with only 1 histologic risk factor. (Reviewer-T. David Bourne, MD).

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Keywords: Morphologic Features, Molecular Features, Pilocytic Astrocytoma

Print Tag: Refer to original journal article
Salivary Gland Secretory Carcinoma Described for the First Time


Skálová A, Vanecek T, et al:


Tumors analogous to SCs of the breast occur in the salivary glands.

**Background:** Secretory carcinoma (SC) of the breast is an uncommon variant of breast cancer. It is composed of circumscribed nodules of bland neoplastic epithelial cells that are arranged in microcystic, tubular, and solid growth patterns. Tumor cells typically have low-grade nuclei with pink granular or vacuolated cytoplasm. Typically they are ER, PR, and HER-2/neu negative and are immunoreactive with antibodies to S100, vimentin, and EGFR. The tumors have also been shown to harbor a unique chromosomal translocation, the t(12;15) (p13;q25) that juxtaposes the *ETV6* and *NTRK3* genes. Recently, some have noted that these tumors are similar to tumors of the breast that have been designated as acinic cell carcinoma.

**Objective:** To describe a series of similar appearing tumors of the salivary glands.

**Methods:** 16 salivary gland tumors with histologic features similar to those of breast SCs were identified at multiple institutions. Thirty other salivary gland tumors were used as controls, as were 3 cases of SC of the breast. Immunohistochemistry was performed with numerous antibodies. RT-PCR was performed for the *ETV6-NTRK* fusion transcript and fluorescence in situ hybridization (FISH) was performed with a break-apart probe to the *ETV6* gene.

**Results:** Patients ranged in age from 21 to 75 years, and 9 patients were men. Thirteen cases occurred in the parotid and 3 within the oral tissues. Tumors had originally been diagnosed as acinic cell carcinomas, adenoid cystic carcinomas, cystadenocarcinomas, and adenocarcinomas, not otherwise specified. They ranged in size from 0.7 to 5.5 cm. Of the 15 cases with follow-up, 3 had recurrences, 2 with metastases. The tumors were rubbery and white-tan. They were circumscribed with nodules of tumor cells separated by fibrous septa. Extension into the salivary gland and surrounding tissues was frequently seen. Tumor cells were arranged in microcystic, tubular, and solid growth patterns. Nuclei were vesicular with fine chromatin and distinct central nucleoli. Pink granular and vacuolated cytoplasm was seen. Atypia was mild, and mitotic figures were rare. Perineural invasion, vascular invasion, and necrosis were not seen. A bubbly, PAS-positive secretion was seen in the microcystic and tubular spaces. Serous differentiation was not seen. Tumors cells were typically immunoreactive with antibodies to CK7, CK8, CK18, CK19, GCDFP-15, EMA, STAT5a, mammaglobin, and S100. Basal or myoepithelial markers were typically nonreactive. All but 1 case showed the *ETV6-NTRK3* fusion transcript by PCR, and all showed the broken *ETV6* gene by FISH.

**Conclusions:** This series describes, for the first time, mammary analogue SC of the salivary gland. These tumors are uncommon and are low-grade malignancies.

**Reviewer's Comments:** With the better understanding of chromosomal rearrangements of salivary gland tumors, we are better able to distinguish some tumors that were previously lumped together. FISH or other molecular testing may someday be routine with these tumors. (Reviewer-Erward B. Stelow, MD).

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Keywords: Secretory Carcinoma, Parotid, *ETV6-NTRK3* Translocation, Immunohistochemistry

Print Tag: Refer to original journal article
Is Interval Breast Carcinoma More Aggressive?

Aggressiveness of ‘True’ Interval Invasive Ductal Carcinomas of the Breast in Postmenopausal Women.

van der Vegt B, Wesseling J, et al:

Mod Pathol 2010; 23 (April): 629-636

As defined, ‘true’ interval breast carcinomas are larger and have a shorter disease-free survival than screen-detected/missed carcinomas, but overall survival is not different.

**Background:** Interval breast cancer describes a clinically symptomatic breast carcinoma detected between the intervals of screening events. Some have described interval carcinomas as more aggressive and have suggested different treatment protocols; however, a complete comparison to screen-detected carcinomas has been limited by heterogeneous study designs.

**Objective:** To compare a strictly defined group of interval breast carcinomas to a similar group of screen-detected carcinomas.

**Methods:** Only postmenopausal women who actively participated in the biannual breast screening program and were surgically treated for breast cancer were included for further evaluation. Each mammogram was reviewed for a consensus reading, and few cases were excluded because the screening mammogram was not available. The breast tumors were classified as: **screen-detected** if detected by the screening mammogram; **missed** if retrospective study review showed a visible lesion on the screening mammogram (false-negative); and **interval** if a clinically detected carcinoma occurred between screening mammograms, but no lesion was noted on retrospective review. Each tumor was incorporated into a tissue microarray, which was subjected to immunohistochemistry for estrogen receptor (ER) and progesterone receptor (PR), Her2/neu, and p53. Clinicopathologic data were compared between the interval carcinomas and screen-detected/missed carcinomas.

**Results:** 23 women presented with a clinically detected breast carcinoma after a negative screening mammogram; 7 of these were missed carcinomas because a visible lesion was found on retrospective review of the screening mammogram, and 16 were ‘true’ interval carcinomas. On univariate analysis, interval carcinomas had a larger tumor size and were more likely to be negative for ER than were the screen-detected or missed carcinomas. On multivariate analysis, only the larger size of interval carcinomas reached statistical significance. A Cox regression analysis showed a trend for shorter time to relapse for the interval carcinomas, but no difference in overall survival was noted.

**Conclusions:** True interval breast carcinomas are clinically detected within the screening interval in women actively participating in a screening program. In addition, no visible lesion is noted on retrospective review of the screening mammogram (false-negative mammogram). In a postmenopausal group, the interval breast cancers were larger than the screen-detected/missed cancers and had a shorter relapse-free survival, but overall survival was not different.

**Reviewer’s Comments:** The authors strictly applied criteria for women with interval carcinoma by excluding those who failed to actively participate in a screening program and those whose mammograms were misinterpreted. There seems to be minimal differences when defined as above. (Reviewer-Mary T. Galgano, MD).

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Keywords: Breast Cancer, Interval- vs Screen-Detected

Print Tag: Refer to original journal article
Some histologic features help predict malignant features with epithelioid angiomyolipomas.

**Background:** Angiomyolipomas (AMLs) are uncommon mesenchymal tumors of the PEComa family that occur most often in the kidney. They are almost exclusively benign and lymph node involvement is typically considered multifocal growth rather than metastasis. Some of these tumors have prominent epithelioid cells. This morphology is seen in clinically benign AMLs and some cases that behave in a more aggressive fashion. Thus the authors of this manuscript divide their epithelioid AMLs into those without and with atypia, believing that the latter category is associated with malignant potential.

**Objective:** To report the histologic spectrum and biologic behavior of 40 consecutive cases of epithelioid AMLs with atypia and to assess whether cases can be stratified prognostically based on clinical and pathologic features.

**Methods:** Epithelioid AMLs with atypia were collected from 3 institutions, and all slides were reviewed. Atypical epithelioid cells were defined as atypical polygonal cells with abundant cytoplasm, vesicular nuclei, prominent nucleoli, and nuclear size that was more than twice as large as adjacent nuclei. The degree of atypia was further classified as moderate or severe. Gross and histologic features were recorded, and follow-up was obtained.

**Results:** There were 40 cases. The mean age was 50.5 years, and the female-to-male ratio was 1.6:1. The average tumor size was 7.2 cm. The mean percentage of epithelioid component was 68%. The mean percentage of epithelioid cells with nuclear atypia was 58%, and a majority of cases had severe nuclear atypia. Multinucleated giant cells and necrosis were present in 55% and 38% of cases, respectively. Mitotic figures ranged from 1 to 6 per 10 high-powered fields (hpf). Lymphovascular invasion was present in 6 cases, and hilar and perinephric fat involvement was present in 5 and 6 cases, respectively. Twenty-six percent of cases were malignant, and of those cases, almost half resulted in the death of the patients. Older patient age, larger tumor size, higher percentage of epithelioid component, severe atypia, higher percentage of atypical cells, higher mitotic count, atypical mitotic figures, necrosis, lymphovascular invasion, and renal vein invasion were all associated with malignant behavior. The authors found that the presence of at least 3 of the following features was associated with malignancy: (1) >70% atypical epithelioid cells, (2) two or more mitotic figures per 10 hpf, (3) atypical mitotic figures, and (4) necrosis.

**Conclusions:** The authors propose that their model for malignant behavior for epithelioid AMLs can be helpful for stratifying risk.

**Reviewer's Comments:** Here the authors report a large series of epithelioid renal AMLs with atypia. After correctly diagnosing these cases, pathologists will now have to spend a bit more time describing the particular histologic features of these tumors. (Reviewer-Edward B. Stelow, MD).

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**Keywords:** Angiomyolipoma, Epithelioid, Atypia, Malignancy

**Print Tag:** Refer to original journal article
Fixation times (1 to 9 hours) may not have an impact on IHC ER expression.

**Background:** In 2008, consensus guidelines for estrogen receptor (ER) immunohistochemistry (IHC) testing for breast cancer were published. One important consideration was that ER immunohistochemical analysis was said to vary with the time of fixation, and 8 hours was recommended as the minimum fixation time for core biopsy specimens.

**Objective:** To examine length of fixation in 10% buffered formalin on the IHC expression of ER in breast tissue.

**Methods:** Small sections (4 x 4 x 2 mm) of tissue were taken from lumpectomy and mastectomy specimens upon receipt in the laboratory. All cases were known to be high expressing ER tumors. The tissue was immediately placed in 10% buffered formalin for either 1, 3, 6, or 9 to 10 hours before processing using a fast routing protocol (2.5 hours) that did not include additional time in formalin. Four slides were prepared, which included 1 H&E and 3 slides that were incubated with anti-ER. There were 3 different anti-ER antibodies evaluated in this study (1 each from SP1-Ventana, 1D5-Zymed, and 6F11-Novocastra). The slides were reviewed by 4 pathologists, and the stains were evaluated for intensity of nuclear staining (1+, weak; 2+, intermediate; and 3+, strong) and percentage of positive tumor cells.

**Results:** All tumors consisted of invasive carcinomas (ductal or lobular) with high expression of ER. There was no significant staining difference between the various antibodies and among the various times of fixation. Specifically, all cases had the same intensity and percentage of nuclear staining with ER. This manuscript includes 5 pages of images illustrating these results.

**Conclusions:** It is recognized that a number of factors can affect ER IHC expression, including the fixative, thickness of the specimen, time to immersion, antibody used, and antigen retrieval process, as well as method of tissue processing. There have been data to show differences in ER expression in the tissue biopsy and subsequent excision, and proper fixation and sampling error likely contribute to this. However, this study shows that with other variables being controlled for, there is consistency in ER staining in specimens, even with variation in fixation time (1 hour minimum).

**Reviewer’s Comments:** This is a small study, but it raises important points and the need for more extensive studies to develop scientific evidence in an effort to standardize testing procedures, not just with ER immunohistochemistry. As an example, in this study, rapid processors were used that are not recommended by the consensus group; however, the data argue against that here. (Reviewer-William A. Kanner, MD).

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Keywords: Breast Cancer, ER, Fixation Time

Print Tag: Refer to original journal article
Serrated Lesions of the Appendix Do Occur

Serrated Lesions of the Appendix: A Morphologic and Immunohistochemical Appraisal.

Bellizzi AM, Rock J, et al:

Am J Clin Pathol 2010; 133 (April): 623-632

Serrated appendiceal lesions do occur and show some morphologic and immunophenotypic similarities with their colorectal counterparts.

Background: Separate from the adenoma-carcinoma pathway in colorectal carcinoma is the serrated pathway. In this sequence, a sessile serrated adenoma develops overt cytologic dysplasia, ultimately resulting in a microsatellite unstable adenocarcinoma. Serrated polyps have also been studied with various immunohistochemical (IHC) stains, including CK20, Ki-67, gastric pyloric mucin MUC6, and β-catenin). Serrated appendiceal lesions pose a dilemma with diagnostic terminology; also, much less is known about these lesions.

Objective: To characterize a series of serrated appendiceal lesions by morphology and immunohistochemistry.

Methods: The case files at Ohio State University were searched for possible serrated appendiceal lesions. All cases were then reviewed by 4 pathologists and reassigned by consensus to the following diagnostic categories: hyperplastic polyp (HP); sessile serrated adenoma (SSA); mixed serrated and adenomatous lesion (MSAL); mucinous cystadenoma (MCA) or conventional adenoma (CAD). Immunohistochemistry with the above stated markers was also completed.

Results: There were 6 HPs, 12 SSAs, 16 MSALs, 14 MCAs, and 2 CADs. In 3 cases, a consensus diagnosis could not be reached (HP vs SSA). Histologically, HPs tended to involve a portion of the appendiceal circumference with superficial epithelial serration tapering down to a narrow base and lacked cytologic dysplasia. SSAs were circumferential in nature, had some combination of crypt dilatation, branching, transverse-lying crypts, deep serration, mucous cells in crypt bases, and also lacked cytologic dysplasia. MCAs were circumferential with dysplastic mucinous epithelial lining that was predominantly flat or gently undulating. By IHC, HPs had expanded (staining beyond the upper quarter of the crypt height) CK20 staining, and half demonstrated expanded Ki-67. MUC6 staining was noted in only 1 case. SSAs were similar with 2 differences: more cases demonstrating expanded Ki-67 and all cases with MUC6 staining. Also, the staining was irregular (random strong expression in the deep crypts). MSALs had similar CK20 profile, but Ki-67 staining was more haphazard, and MUC6 was seen in half of the cases. MCAs had diffuse CK20, haphazard Ki-67 labeling, and no MUC6 expression. The 2 CADs demonstrated expanded/irregular CK20, expanded Ki-67, and were negative for MUC6. β-catenin was expressed in all lesions except for 1 CAD.

Conclusions: There are serrated lesions of the appendix that have morphologic similarities to those lesions found in the colorectum. The IHC profiles are also similar and may help in further classifying the lesion. Although molecular data thus far indicate that these are different, this is an area that is still incomplete.

Reviewer's Comments: Although molecular data call into question an analogous serrated pathway of appendiceal neoplasia, there are recognized serrated lesions of the appendix that warrant further study. If one encounters these lesions, a descriptive diagnosis may be rendered with an accompanying note addressing the unclear nature of these lesions at the present time. (Reviewer-William A. Kanner, MD).

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Keywords: Appendix, Adenomas, Serrated

Print Tag: Refer to original journal article
Epithelioid Sarcomas Express EGFR Without Amplification/Mutation

Epithelioid Sarcoma Expresses Epidermal Growth Factor Receptor but Gene Amplification and Kinase Domain Mutations Are Rare.

Cascio MJ, O'Donnell RJ, Horvai AE:
Mod Pathol 2010; 23 (April): 574-580

Epithelioid sarcomas express EGFR but lack gene amplification or mutation. Response to targeted therapy has not been established.

**Background:** Therapy directed at inhibiting the epidermal growth factor receptor (EGFR) can be used in carcinomas with aberrant signaling, particularly colorectal, lung, and head and neck squamous cell carcinomas. Mutations in the tyrosine kinase domain of EGFR or amplification are predictors of response to this targeted therapy. More recently, however, sarcomas have been described as having expression of the EGFR protein with a subset of those showing molecular alterations, such as amplification or mutation. Epithelioid sarcomas have immunophenotypic similarity with carcinomas and may harbor EGFR alterations that would accommodate targeted therapy.

**Objective:** To characterize expression and molecular alterations of EGFR in epithelioid sarcomas for consideration of targeted therapy.

**Methods:** 15 epithelioid sarcomas representing all 3 subtypes (distal, proximal, and fibroma-like) were collected and subjected to routine diagnostic immunohistochemistry. EGFR pharmDx was also performed and scored according to the manufacturer’s recommendations, specifically—a positive result described as >1% of tumor cells with partial or complete membranous staining of any intensity. Percentage and intensity were scored for the study. Fluorescence in situ hybridization (FISH) was performed using the EGFR and CEP 7 probes and scored as an average signal in 50 cells. Polymerase chain reaction was performed to assess mutational status of EGFR.

**Results:** 14 out of 15 tumors were positive for EGFR pharmDx, and 73% were strong (2+ or 3+) and diffuse (>75% of cells) membranous staining. Fourteen cases evaluated were all negative for amplification or polysomy of the EGFR gene. Twelve cases with successful sequencing were all negative for point mutations, insertions, or deletions involving the EGFR gene.

**Conclusions:** Although most epithelioid sarcomas have strong and diffuse expression of EGFR protein, none harbor molecular alterations of the EGFR gene. Given the need for systemic adjuvant therapy, response to inhibitors should be evaluated in future studies.

**Reviewer’s Comments:** Given the recent description of EGFR+ sarcomas, the authors chose to begin a thorough evaluation of one with epithelial characteristics. In carcinomas, targeted therapy is most likely to be effective in tumors that have activating genetic alterations in addition to protein expression. The clinical significance of this study is still unclear. (Reviewer-Mary T. Galgano, MD).

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Keywords: Epithelioid Sarcomas, EGFR Expression

Print Tag: Refer to original journal article
Expression of HMGA1 correlates with tumor grade and decreased survival from pancreatic ductal adenocarcinomas.

**Background:** Pancreatic ductal adenocarcinoma (PDA) is a highly lethal cancer with few treatment options partially due to the common presentation of locally aggressive or advanced metastatic stage. Precursor lesions, namely pancreatic intraepithelial neoplasia (PanIN), have been characterized with associated molecular alterations, but the driving force behind progression to invasive carcinoma is not known. High-mobility group A1 (HMGA1) protein has been implicated in PDA among many other human cancers and may play a role in tumor progression.

**Objective:** To characterize the expression of HMGA1 in pancreatic cancer and PanIN with correlation to patient outcome.

**Methods:** Cultured PDA cells and normal pancreatic cells were subjected to reverse transcription polymerase chain reaction (RT-PCR) for HMGA1 expression. Tissue microarrays were constructed of PDA and PanIN lesions for immunohistochemical detection of HMGA1 protein for correlation to clinicopathologic features.

**Results:** *HMGA1a* mRNA was increased 4-fold, 7-fold, and 14-fold in the 3 PDA cell lines derived from patients with advanced disease. Of the 125 PDA tumors in the tissue microarray, 123 (98%) were positive for HMGA1 by immunohistochemistry, but 18% of those were limited reactivity. Benign pancreatic ducts were negative, but benign biliary ducts were notably positive. The HMGA1 reactivity was inversely correlated to mean survival and directly correlated to tumor grade. There was no correlation found between expression and lymph node metastases or overall stage. Of the PanIN lesions, the staining correlated with increasing grade; 78% of PanIN-3, 55% of PanIN-2, and 36% of PanIN-1 demonstrated immunoreactivity.

**Conclusions:** HMGA1 is overexpressed in PDA compared to normal and may have a role in tumorigenesis. The presence of protein expression is noted to correlate with increasing severity of PanIN lesions and correlates to poor prognostic factors in PDA.

**Reviewer's Comments:** Given the presence of HMGA alterations in a variety of human cancers, overexpression may provide a valuable therapeutic target or prognostic biomarker in advanced disease. (Reviewer-Mary T. Galgano, MD).

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Keywords: Ductal Neoplasia, HMGA1 Expression; Tumor Grade, Survival

Print Tag: Refer to original journal article
The presence of a high-grade spindle cell component is associated with an increased risk of distant metastasis and poor patient outcome.

**Background:** Metaplastic breast carcinoma is a rare subtype of breast cancer that contains a nonglandular component. The metaplastic components can be epithelial (e.g., squamous), mesenchymal (e.g., spindle cell, osteocartilaginous, matrix producing), or a mixture of cell types. Metaplastic carcinomas are known to have a worse prognosis than conventional breast carcinomas, despite the tendency to less frequently have lymph node metastasis. Several studies have attempted to create a meaningful histologic classification of metaplastic breast carcinomas, with limited success in predicting patient outcomes.

**Objective:** To evaluate the significance of spindle cell elements on outcome in patients with metaplastic breast carcinoma.

**Methods:** The archives of 1 institution were retrospectively searched over 20 years for patients with a diagnosis of metaplastic breast carcinoma. The surgical resections were reviewed by 3 independent pathologists and reclassified according to the presence or absence of a spindle cell component. Cases that contained a spindle cell component were further subdivided according to high- or low-grade features (nuclear atypia, increased mitotic activity, and hypercellularity). All cases were also assessed for the presence of squamous, heterologous osteocartilaginous, and matrix elements. All patients had at least 1.5 years of follow-up, with a mean of 5.3 years. Distant metastasis was considered the end point for outcome analysis.

**Results:** 53 patients were diagnosed with metaplastic breast carcinoma during the study period, of which 15 (28%) developed distant metastasis. Twenty-four cases (45%) had a spindle cell component; 6 demonstrated spindle cells only, and 18 contained spindle cells and at least 1 other metaplastic element. Twelve cases (50%) were considered high grade. Squamous elements were present in 33 cases (62%), of which 22 were pure squamous cell carcinoma, and 11 were associated with spindle cell elements. Seven cases (29%) with a spindle cell component developed distant metastasis, all of which were high grade. Eight cases (28%) that did not have a spindle cell component developed metastasis, all of which were pure squamous cell carcinoma. High-grade spindle cells or a pure squamous cell component were independent predictors of outcome when compared with tumor size and presence of axillary lymph node metastases.

**Conclusions:** The presence of high-grade spindle cells is associated with an increased risk of distant metastasis and poor patient outcome. When present, this finding should be reported for appropriate patient triage and potentially more aggressive therapy.

**Reviewer's Comments:** Previous studies have suggested that heterologous elements may be associated with a worse prognosis. In this study, all patients with heterologous elements and metastasis also had a high-grade spindle cell component. This is a promising criterion to identify high-risk metaplastic breast carcinoma patients. (Reviewer-Deborah J. Chute, MD).

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Keywords: Metaplastic Breast Carcinoma, Squamous Cell Carcinoma, Spindle-Cell Carcinoma, Matrix-Producing Carcinoma, Prognostic Factor

Print Tag: Refer to original journal article
Background: Lung cancer is the most common cancer worldwide. The most powerful predictors of prognosis are tumor stage and tumor grade. While there is a well-defined system for staging pulmonary carcinomas, the criteria for grading tumors remain nebulous. Some authors have suggested using nuclear morphometry to better quantitate tumor grading, but it has not gained widespread use. In some organ systems, such as breast cancer, nuclear grade is independently scored from the overall grade.

Objective: To evaluate the utility of nuclear morphometry in identifying pulmonary adenocarcinomas with a more favorable prognosis.

Methods: Patients with pulmonary adenocarcinomas ≤2 cm who were treated surgically and had follow-up clinical information were included. Patients with neoadjuvant chemotherapy or radiotherapy were excluded. The resected specimens were fixed in 10% formalin and sections stained with H&E for light microscopy. Morphometric analysis of nuclear size (area, dimension, and roundness) was performed by 4 independent pathologists on 50 cells per tumor from areas with the largest tumor nuclei. Small lymphocyte size was used as an internal standard. Tumors were judged to have increased nuclear size when the nuclear area was 5x larger than the internal control lymphocytes in at least 3 fields. Nuclear size was correlated with other clinicopathologic characteristics, including tumor stage, adenocarcinoma subtype, and lymph node metastasis.

Results: 133 patients met the inclusion and exclusion criteria, with a mean follow-up of 80 months. Fifty-four of the tumors (41%) had increased nuclear size. On univariate analysis, increased nuclear size was significantly associated with reduced overall survival; there was >90% survival at 5 years for tumors with small nuclear area compared to <60% survival at 5 years for tumors with high nuclear area. On multivariate analysis, increased nuclear size was significantly associated with increasing tumor stage, pleural invasion, and lymph node metastasis.

Conclusions: Nuclear size and area may be a useful prognostic marker for evaluating pulmonary adenocarcinomas, particularly in low-stage tumors.

Reviewer's Comments: While this study has little direct application to practicing pathologists (few of whom will ever use nuclear morphometry), it suggests that providing a nuclear grade in pulmonary adenocarcinomas may be useful in predicting prognosis, similar to the use of a separate nuclear grade in breast carcinomas. Until now, we have been using nuclear features in the overall grading of pulmonary adenocarcinomas either consciously or subconsciously. (Reviewer-Deborah J. Chute, MD).

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Keywords: Nuclear Grading, Prognosis, Pulmonary Adenocarcinoma, Nuclear Area, Nuclear Diameter

Print Tag: Refer to original journal article
Small Cell Carcinoma vs Urothelial Carcinoma of the Bladder

Inverse p16 and p63 Expression in Small Cell Carcinoma and High-Grade Urothelial Cell Carcinoma of the Urinary Bladder.

Buza N, Cohen PJ, et al:


For the most part, p16 and p63 show inverse staining patterns in small cell (p16+/p63-) versus high-grade urothelial cell (p16-/p63+) bladder carcinomas.

Background: Although rare, small cell carcinoma (SmCC) of the urinary bladder occurs in patients with demographic characteristics similar to those with high-grade urothelial carcinoma (HG-UCC). However, stage for stage, SmCC is a more aggressive tumor that is associated with worse clinical outcomes than HG-UCC. Current diagnostic criteria proposed by the World Health Organization (WHO) utilize morphologic features alone to support a diagnosis, since standard neuroendocrine markers may be negative in small cell bladder cancers. Thus, studies are still being performed in an attempt to identify a single immunohistochemical stain or stain panel that can help distinguish between these 2 tumor types.

Objective: To determine whether a panel of immunohistochemical markers including p16 and p63 helps distinguish SmCC from HG-UCC of the urinary bladder.

Methods: 14 cases of SmCC of the urinary bladder and 16 cases of HG-UCC of the urinary bladder were retrieved. Slides from all cases were reviewed, and a representative block from each case was submitted for immunohistochemical staining using a panel of stains (p16, p63, CK20, CK7, chromogranin, synaptophysin, and CD56). p16 and p63 positivity was defined as staining of at least 10% of tumor cell nuclei. Positivity for all other markers was accepted even if only rare tumor cells showed appropriate cytoplasmic and/or membrane staining.

Results: p16 expression was observed in 43.7% of HG-UCCs and 92.8% of SmCCs. In contrast, p63 was positive in a majority of HG-UCCs (81.3%) compared to SmCCs (7.1%). CK7 and CK20 were positive in 64.3% and 14.3% of SmCCs, and in 81.3% and 50.0% of HG-UCCs, respectively. The neuroendocrine markers chromogranin, synaptophysin, and CD56 were positive in 28.6%, 64.3%, and 71.4% of SmCCs, respectively. Among HG-UCCs, only rare cells showed positivity for synaptophysin (6.3%).

Conclusions: The authors suggest that a helpful immunohistochemical marker profile for SmCC of the bladder includes a p16+, p63-, CK20- result, while that of HG-UCC includes a p16+/-, p63+, CK20+ result. The authors conclude that p16 positivity in the absence of CK20 and p63 positivity strongly supports SmCC, while CK20 and p63 positivity with or without p16 staining supports an HG-UCC diagnosis.

Reviewer's Comments: Although one encounters SmCC of the bladder infrequently, the overlapping features with very high-grade UCC make the distinction between these 2 tumor types difficult at times. The inclusion of various neuroendocrine markers would still seem prudent, and the addition of uroplakin to the panel of immunostains may also add discriminatory power. (Reviewer-T. David Bourne, MD).

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Keywords: High-Grade Bladder Carcinoma, Immunohistochemistry

Print Tag: Refer to original journal article
The gene and protein expression profiles of hepatocellular carcinomas arising in cirrhosis differ from the profiles of those tumors occurring in non-cirrhotic livers.

**Background:** While hepatocellular carcinoma most commonly arises in the setting of cirrhosis (HCC-C), approximately 10% of cases are seen in patients without cirrhosis (HCC-NC). Although the etiology of HCC-NC is unclear in some cases, causative factors appear to include chronic HBV infection (especially in children), chemical exposures, and metabolic disorders. Various molecular abnormalities have been identified in HCC, and they have been broadly classified into 4 main groups: chromosomal gains and losses; p53 pathway alterations; Wnt/β-catenin pathway alterations; and retinoblastoma pathway (Rb1) alterations.

**Objective:** To compare the expression of genes involved in the p53, Wnt/β-catenin, and Rb1 pathways in HCC-C versus HCC-NC, and to validate any protein expression using immunohistochemistry.

**Methods:** In contrast to HCC-C, HCC-NC was defined by features of HCC arising in liver tissue showing a lack of bridging fibrosis. Laser microdissection was used to capture tissue samples from 78 cases (normal liver, 20; cirrhotic liver, 20; HCC-C, 20; and HCC-NC, 18). Gene expression profiles were then analyzed using an Affymetrix GeneChip oligonucleotide array, which included probe sets for p53, β-catenin, Rb, the cell cycle regulators p16INK4, p21Waf1/cip1, and p27Kip1, and various cyclin-dependent kinases. Immunohistochemistry using monoclonal antibodies against most of the above protein products was then performed.

**Results:** HCC-C showed a significantly higher rate of p53, cyclin D1, and β-catenin expression than HCC-NC. In contrast, cases of HCC-NC showed significantly higher rates of p21Waf1/cip1 and p27Kip1 expression. No significant association between tumor grade, lymphovascular invasion, or underlying etiology and genetic marker expression was observed. Large tumors showed p16INK4 and p21Waf1/cip1 expression compared with small tumors. Aberrant β-catenin expression was more commonly seen in cases with single, rather than multiple, tumors.

**Conclusions:** Both gene and protein expression analysis support the conclusion that p53 pathway alterations are more commonly seen in HCC-C, whereas alterations in the cell cycle regulatory proteins p21Waf1/cip1 and p27Kip1 appear to play a more important role in the pathogenesis of HCC-NC.

**Reviewer’s Comments:** The study provides data to support one’s intuitive guess that HCC-C and HCC-NC likely have different underlying genetic determinants. While the findings may not be readily applicable to one’s daily case sign-out, the knowledge as it relates to HCC pathogenesis is helpful. (Reviewer-T. David Bourne, MD).

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Keywords: Hepatocellular Carcinoma, Cirrhosis, Non-Cirrhosis

Print Tag: Refer to original journal article
Endoscopic ultrasound-fine-needle aspiration has a false-positive rate of more than 5% at some institutions.

**Background:** Endoscopic ultrasound (EUS) is used typically for the diagnosis and staging of malignancy. When coupled with fine-needle aspiration (FNA), it allows for the cytologic sampling of lesions under real-time guidance. Its sensitivity for the diagnosis of malignancy is typically reported to be between 60% and 90%, and most manuscripts present specificities for the technique that approach 100%.

**Objective:** This study investigates one center's experience with false-positive EUS-FNA diagnoses and used root-cause analysis to determine whether error was the result of sampling or interpretive error.

**Methods:** All patients with positive findings or who were suspicious for malignancy diagnoses by EUS-FNA seen at a single institution over nearly 13 years were identified. All patients who then underwent surgical resection of the sampled site who did not receive neoadjuvant therapy were included. False-suspicious and positive diagnoses were defined as cases that were originally diagnosed as positive or suspicious for malignancy, respectively, but were found to be benign at resection. Cases designated false positive and false suspicious were then reviewed to determine whether the error was interpretive.

**Results:** There were nearly 5500 EUS-FNAs performed during the time period. Slightly more than 2500 cases were interpreted as positive, suspicious, or atypical. Of these, 377 patients underwent resection without having received neoadjuvant therapy, representing only 6.7% of patients who underwent EUS-FNA. Indications for EUS-FNA included the staging of luminal cancer (21%), the diagnosis of pancreatic neoplasia (61%), and the identification of other lesions. The false-suspicious and positive rate combined was 7.2%, and the false-positive rate alone was 5.3%. More than 80% of these cases were non-pancreatic FNAs. Almost all of these cases were sampled nodes in patients with suspected or proven luminal malignancy who were found to have node-negative malignancies at surgery. By review, it was determined that 50% of the incorrect positive or suspicious diagnoses were due to sampling, and 50% were due to interpretation.

**Conclusions:** The authors suggest that false-positive diagnoses are not as uncommon with EUS-FNA as once thought.

**Reviewer's Comments:** The authors state that by using actual histologic follow-up rather than clinical follow-up, they have improved the accuracy of their study. This is only true, however, if false-positive patients are not more likely to undergo surgery than true-positive patients. (Reviewer-Eduard B. Stelow, MD).

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Keywords: Endoscopic Ultrasound, Fine-Needle Aspiration, False Positive, Accuracy

Print Tag: Refer to original journal article
PAX8 Helpful in Serous Neoplasia

PAX8 Reliably Distinguishes Ovarian Serous Tumors From Malignant Mesothelioma.

Laury AR, Hornick JL, et al:

Am J Surg Pathol 2010; 34 (May): 627-635

PAX8 is a very helpful immunostain for distinguishing serous carcinoma and mesothelioma.

**Background:** It can sometimes be very difficult to distinguish serous carcinomas of the ovary and peritoneum from malignant mesothelioma of the abdominal cavity. Although a number of studies have been published using panels of immunohistochemical markers for this purpose, few stains have proved particularly helpful. For example, although calretinin, WT-1, podoplanin, and mesothelin are typically expressed by mesotheliomas, they are also expressed not infrequently by serous carcinomas. Other more specific markers of glandular differentiation, such as B72.3, tend to be less sensitive. Recently, estrogen receptor and h-caldesmon have been suggested as markers for serous carcinomas. PAX8 is a transcription factor important in organogenesis of the Müllerian system. It is a nuclear marker and has been shown to be expressed by ovarian serous carcinomas.

**Objective:** To investigate the use of PAX8 for distinguishing serous carcinomas and mesotheliomas.

**Methods:** 304 cases were obtained from a single institution for immunohistochemistry. These included 23 peritoneal epithelioid mesotheliomas, 2 peritoneal well-differentiated papillary mesotheliomas, 1 peritoneal multicystic mesothelial inclusion cyst, 24 pleural epithelioid mesotheliomas, 92 serous borderline tumors, 10 low-grade serous carcinomas, and 152 high-grade serous carcinomas. Immunohistochemistry was performed with antibodies to PAX8 and h-caldesmon and was scored based on intensity and extent.

**Results:** 99% of high-grade serous carcinomas and 100% of low-grade serous neoplasms were immunoreactive with antibodies to PAX8. Diffuse immunoreactivity was seen in 86% of the serous borderline tumors, mostly with intense staining. Sixty-six percent of the high-grade serous carcinomas were diffusely immunoreactive with antibodies to PAX8, although approximately half of these showed moderate to weak intensity; h-caldesmon was nonreactive with all serous neoplasms. All pleural mesotheliomas were negative for PAX8, and only 9% of the peritoneal cases showed focal or weak staining. All 3 well-differentiated papillary mesotheliomas were immunoreactive with antibodies to PAX8, as was the multicystic inclusion cyst; h-caldesmon was nonreactive with the majority of mesothelial neoplasms in this study.

**Conclusions:** PAX8 is a very helpful immunostain for distinguishing peritoneal mesothelioma from serous carcinoma; h-caldesmon was not a good marker in this study.

**Reviewer's Comments:** It may be helpful to add PAX8 to one's battery on immunostains for distinguishing mesotheliomas from adenocarcinomas. It would be interesting to know what percentage of adenocarcinomas other than ovarian serous carcinomas are immunoreactive with this antibody. (Reviewer-Edward B. Stelow, MD).

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Keywords: Serous Carcinoma, Mesothelioma, Immunohistochemistry, PAX8, Caldesmon

Print Tag: Refer to original journal article