How Should Laboratories Report CEP17 Copy Number Gain in Breast Cancer?

HER2 In Situ Hybridization in Breast Cancer: Clinical Implications of Polysomy 17 and Genetic Heterogeneity.

Hanna WM, Rüschoff J, et al:

Mod Pathol 2013; June 28 (): epub ahead of print

For breast cancers with mean gain in CEP17 copy number (polysomy), the mean HER2 copy number and immunohistochemical score should be considered as well as the HER2:CEP17 ratio.

Discussion: When measuring breast tumors for HER2 gene amplification, an increased number of centromere 17 (CEP17) signals detected by in situ hybridization (ISH; mean >3 signals per nucleus) has been incorrectly presumed to reflect whole chromosome polysomy. Instead, it most often represents pericentromeric amplification. Occurring in roughly 25% to 30% of breast cancers (reported prevalence range, 3% to 46% of tumors), CEP17 copy gain in the absence of HER2 amplification has been inconsistently associated with unfavorable pathologic features, higher prevalence of equivocal 2+ immunohistochemical (IHC) scoring, and higher prevalence of discordant 3+ IHC scoring. Occasional documented cases of IHC 3+, non-amplified breast cancers with CEP17 copy gain (polysomy) have responded to trastuzumab therapy. In addition, there is inconsistent evidence for IHC 0/1+/2+, non-amplified breast cancers with CEP17 copy gain (polysomy) responding to trastuzumab therapy. Since HER2 genetic heterogeneity is inconsistently defined and reported, its clinical significance is poorly understood. Standardized rules for ISH scoring and concordance of definitions for ISH- and IHC-based heterogeneity are required, but already there is evidence that significant levels of HER2 heterogeneity affect disease-free survival and speculation that it may impact eligibility for trastuzumab.

Recommendations: For breast cancers with mean gain or loss of CEP17 copy number, mean HER2 copy number and IHC score should be considered as well as the HER2:CEP17 ratio. IHC3+ tumors and those with HER2 copy number >6 should be considered positive irrespective of a HER2:CEP17 ratio that is affected by aberrant CEP17 copy number.

Reviewer's Comments: This is an outstanding update on clinical implications of (CEP 17 signal gain or polysomy 17) in breast cancer. It includes highly sensible recommendations for reporting the small subset of tumors with HER2:CEP17 ratios that are affected by CEP17 copy number gain. Notably, this review does not address less common loss of CEP17 signal, which is generally described as monosomy 17 and probably occurs in <2% of breast cancers. In our practice, we adopt essentially the same recommendation for reporting those cases by considering IHC staining and HER2 copy number irrespective of an affected HER2:CEP17 ratio. The authors propose their own rules for reporting HER2 heterogeneity. However, since clinical implications of heterogeneity are so poorly understood I feel that specific reporting recommendations are premature. (Reviewer-Guy E. Nichols, MD, PhD).

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Keywords: Breast Cancer, Chromosome 17, Genetic Heterogeneity, HER2 Testing, In Situ Hybridization, Polysomy

Print Tag: Refer to original journal article
What Panel of Stain Should Be Performed for Lung Cancer Diagnosis?

Immunohistochemistry in the Differential Diagnostics of Primary Lung Cancer: An Investigation Within the Southern Swedish Lung Cancer Study.
Brunnström H, Johansson L, et al:
Am J Clin Pathol 2013; 140 (July): 37-46

A panel of cytokeratin 5, thyroid transcription factor 1, and napsin A is of great utility in differentiating adenocarcinoma and squamous cell carcinoma in lung cancer.

**Background:** The histologic type of lung cancer is important for prognosis and treatment modalities, ie, it is not good enough to diagnose small-cell and non–small-cell lung carcinoma anymore. Making this more difficult, more and more specimens consist of small biopsies or cytology specimens. Thus, immunohistochemistry is of utility in cases with unclear morphology.

**Objective:** To evaluate a range of lung cancers with a variety of immunohistochemical stains to assess their utility.

**Methods:** Tissue from patients with various lung cancers was obtained and studied. Patients who had neoadjuvant chemotherapy were excluded. The authors used a tissue microarray in this study, but they note that the size of the tissue is comparable to a small biopsy specimen. Multiple immunostains were tested. Positive staining was if ≥1% tumor cells stained (with 1% to 10% being classified as 1+ and >10% 2+).

**Results:** There were 209 tumors studied: 58% adenocarcinomas, 31% squamous cell carcinomas (SCCs), 7% large-cell carcinomas, 2% adenosquamous cell carcinomas, and 1% sarcomatoid carcinomas. There was also 1 case of small-cell carcinoma. All tumors were positive for broad-spectrum cytokeratins (CKAE1/3 or CKMN). All adenocarcinomas (including the glandular component of adenosquamous cell carcinomas) demonstrated cytokeratin 7 positivity. All but 1 case of SCC stained with both cytokeratin 5 (CK5) and p63 (the case that did not stain was poorly differentiated). One case of adenocarcinoma and 1 large-cell carcinoma demonstrated staining with CK5 and 1% to 2% of tumor cells stained. Estrogen receptor was occasionally positive in both adenocarcinomas and SCCs. Neuroendocrine markers were positive in those cases with the appropriate morphology. Almost all adenocarcinomas (94%) were positive for either thyroid transcription factor 1 (TTF-1) or napsin A. Because of the small number of small-cell and sarcomatoid cases, no definitive conclusions could be made about those cases.

**Conclusions:** In this study, CK5 was a very sensitive marker for SCC and a more specific marker than p63, and the combination of the 2 was not more informative than a single marker. TTF-1 and napsin A were very sensitive for adenocarcinoma, and the combination of the 2 stains was an even more sensitive test for adenocarcinoma.

**Reviewer’s Comments:** I am clearly noting the trend on attempting to distinguish between adenocarcinoma and SCC from lung specimens that are either biopsies or cytology specimens. We currently use a panel that includes CK5/6, CK7, TTF-1, p63, and napsin A and I sometimes add a mucicarmine stain as well. This paper supports this panel, but one must also recognize that there may be tumor heterogeneity in a small sample and the evaluated tissue might not be completely representative. (Reviewer-William A. Kanner, MD).

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Keywords: Adenocarcinoma, Squamous Cell Carcinoma, Cytokeratin 5, Thyroid Transcription Factor 1, Napsin A

Print Tag: Refer to original journal article
PCR More Specific Than Immunohistochemistry for CMV Diagnosis in Placentas

Diagnosis of Congenital CMV Using PCR Performed on Formalin-Fixed, Paraffin-Embedded Placental Tissue.
Folkins AK, Chisholm KM, et al:

Am J Surg Pathol 2013; June 20 (): epub ahead of print

Polymerase chain reaction on archival placenta tissue is as specific as urine culture and more specific than immunohistochemistry for the diagnosis of congenital cytomegalovirus infection.

**Background:** A diagnosis of congenital cytomegalovirus (CMV) infection is contingent upon the detection of the virus within a newborn's first 21 days of life, yet the symptoms of congenital CMV often do not manifest until much later. Clinical complications of congenital CMV include sensorineural hearing loss, developmental delay, ophthalmologic defects, microcephaly, psychomotor impairment, cerebral palsy, and seizures. The majority – up to 90% – of infants infected with CMV will be asymptomatic at birth, but 5% to 15% of these asymptomatic babies will go on to develop permanent defects. Early intervention with ganciclovir, however, has been shown to curb this process. Therefore, it is clinically useful to be able to detect CMV in archival specimens from patients in whom infection was not initially considered. This can be accomplished through immunohistochemistry for CMV on formalin-fixed, paraffin-embedded (FFPE) placental tissue blocks; however, the sensitivity of this technique is debatable.

**Objective:** To evaluate whether polymerase chain reaction (PCR) can reliably detect CMV in FFPE placental tissues.

**Design/Methods:** The Stanford University microbiology database was searched from July 2001 to March 2012 to identify all CMV cultures from infants within the first 100 days of life. Cases of intrauterine fetal demise were also included, as were placentas showing chronic villitis for which urine CMV culture was not performed. All corresponding FFPE placenta samples were then evaluated by H&E to determine the presence, extent, and character (lymphocytic vs lymphoplasmacytic) of chronic villitis, and CMV testing was performed by immunohistochemistry and quantitative PCR.

**Results:** PCR was negative in 100% (20 of 20) of placentas with negative urine CMV cultures and was positive in 100% (5 of 5) cases from infants with positive urine CMV cultures within the first 21 days of life. PCR was also positive in 12.5% (1 of 8) of placentas from infants with first positive urine culture after day 21 of life. In total, 4% (1 of 26) of placentas showing chronic villitis without corresponding urine CMV results were positive by PCR. Of the total 10 placentas positive for CMV by PCR, only 6 were positive by immunohistochemistry.

**Conclusions:** PCR provides a valuable adjunct to histologic and immunohistochemical evaluation of FFPE placental tissue and can help identify newborns requiring close clinical follow-up and older infants and children in whom a diagnosis of congenital CMV was not initially suspected.

**Reviewer's Comments:** Anatomic pathologists have resisted the use of PCR for CMV identification in FFPE tissues due to an assumption that it has low specificity. The results here argue against that concern and parallel what Dr Pinsky and I found in a related study published in July's issue of Am J Surg Pathol, which showed no evidence of false positives in CMV PCR performed on gastrointestinal biopsies. (Reviewer-Anne McGehee Mills, MD).

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Keywords: Cytomegalovirus, Congenital Cytomegalovirus Infection, Sensorineural Hearing Loss

Print Tag: Refer to original journal article
Lewy Bodies Found in Pharyngeal Nerves of PD Patients

Parkinson Disease Affects Peripheral Sensory Nerves in the Pharynx.

Mu L, Sobotka S, et al:

J Neuropathol Exp Neurol 2013; 72 (July): 614-623

Lewy pathology is present in peripheral nerves of pharyngeal tissue in patients who have Parkinson disease and dysphagia.

Background: In addition to the hallmark clinical features of bradykinesia, rigidity, and tremor, patients with Parkinson disease (PD) often suffer from a number of secondary motor symptoms, including dysarthria and dysphagia. This latter problem may eventually lead to aspiration pneumonia, which is the most common cause of death in patients with both PD and dysphagia. While the pathogenesis of dysphagia in the setting of PD is unknown, it has been hypothesized that PD-associated dysphagia may be associated with damage to sensory pharyngeal nerves involved in the initiation or modulation of the swallowing reflex.

Objective: To determine and define the extent of PD-related degenerative changes involving peripheral sensory nerves of the pharynx.

Methods: Pharyngeal tissue was collected at autopsy from patients with clinically diagnosed and pathology confirmed PD (n=10) and from healthy age-matched controls (n=4). From this tissue, sensory nerves were excised to include the glosso-pharyngeal nerve (cranial nerve IX), the pharyngeal sensory branch of the vagus nerve (PSB-X), and the internal superior laryngeal nerve (ISLN) innervating the laryngopharynx. Tissue from each case was then submitted for immunohistochemical staining for α-synuclein in order to detect Lewy pathology.

Results: Tissue from all PD cases showed aggregates of α-synuclein within axons of pharyngeal sensory nerves, while all tissue from control cases was negative with α-synuclein staining. In PD patients with dysphagia, the density of α-synuclein positivity was greater than in PD patients without dysphagia. In addition, nerve fibers from the ISLN showed significantly more α-synuclein staining than those in cranial nerve IX and PSB-X.

Conclusions: In patients with PD, pharyngeal sensory nerves are directly affected by pathologic processes that may result in decreased pharyngeal sensation. This may then lead to swallowing impairment and aspiration via decreased pharyngeal sensation.

Reviewer’s Comments: Some researchers have maintained that dysphagia in the setting of PD is primarily related to a reduction in dopaminergic activity within the basal ganglia. This study, as well as previous studies demonstrating significant α-synuclein pathology within peripheral nerves, suggests that the dopaminergic explanation is not the sole, or even primary, cause of dysphagia in PD patients. (Reviewer-T. David Bourne, MD).

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Keywords: α-Synuclein, Parkinson Disease, Dysphagia, Lewy Bodies, Lewy Neurites

Print Tag: Refer to original journal article
Prognosis in HPV-Associated OSCC -- Are D-Type Cyclins the Answer?

Cyclin D1 - A Prognostic Marker in Oropharyngeal Squamous Cell Carcinoma That Is Tightly Associated With High-Risk Human Papillomavirus Status.

Scantlebury JB, Luo J, et al:

Hum Pathol 2013; 44 (August): 1672-1680

While cyclin D1 may identify a subset of oropharyngeal squamous cell carcinoma with poor prognosis, its association with human papillomavirus may limit its diagnostic utility.

Background: Human papillomavirus (HPV)-related oropharyngeal squamous cell carcinoma (OSCC) is incompletely understood and demonstrates a unique biology with a clinically improved prognosis. While an improved prognosis is expected overall, a subset (10% to 15%) of patients experience progressive disease and distant metastasis. It is known that both cyclin D1 and HPV are associated with tumorgenesis at distinct points in the cell cycle. p16 is a well-known regulator of the D-type cyclins including cyclin D1.

Objective: To evaluate the relationship between cyclin D1 expression, HPV status, and prognosis in OSCC.

Design/Methods: A retrospective review identified a cohort of 202 patients diagnosed with OSCC. Clinical features were obtained from electronic medical records including survival and smoking data. Pathologic features were obtained from pathology reports. All patients were treated with surgery ± postoperative radiation and chemotherapy or were treated with radiation therapy alone ± chemotherapy. Tissue microarrays were constructed from archival paraffin tissue. In situ hybridization (ISH) for high-risk HPV E6/E7 messenger RNA and DNA was performed. Immunohistochemistry was performed for p16 and cyclin D1. Cyclin D1 staining was assessed visually for distribution (% positive cells) and assessed by image analysis for intensity (1+ to 3+). Statistical analysis was performed to identify associations between cyclin D1 and other clinical and pathologic variables.

Results: The majority of patients (90%) presented with lymph node metastasis. The majority (81%) were treated with primary surgery ± postoperative radiation. In total, 80% of patients were p16+ and/or HPV positive by ISH. Of key importance, most HPV RNA+ and/or p16+ OSCC cases had significantly lower cyclin D1 expression. This finding was expected given the relationship between p16 and cyclin D1. Furthermore, low cyclin D1 expression correlated with Caucasian ethnicity, lower smoking rate, positive nodal metastases, and higher overall tumor stage, which are all features of HPV-associated OSCC. The data additionally suggested that cyclin D1 overexpression intensity, not distribution, predicted statistically significant poor overall, disease-free, and disease-specific survival in HPV-associated OSCC.

Conclusions: While cyclin D1 may identify a subset of HPV+ OSCC patients with a poor prognosis, the strong correlation between cyclin D1 and HPV infection may interfere with the clinical utility of cyclin D1.

Reviewer's Comments: Cyclin D1 has been interrogated for prognostic utility in many malignancies, including mantle cell lymphoma. However, cyclin D1 is one member in a diverse family of D-type cyclins that may become upregulated through several mechanisms including epigenetic events. As the ability of HPV to transform cells is so closely related to the cell cycle, substantial confounders exist in the data presented in this article. The authors present their data fairly and are honest about the limitations of cyclin D1 in this setting. (Reviewer-Frank N. Moore, MD).

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Keywords: Human Papillomavirus, Oropharyngeal Squamous Cell Carcinoma, p16, Cyclin D1, Prognosis

Print Tag: Refer to original journal article
A Pitfall With Immunostains for Squamous Cell Carcinoma

Expression of CK7, Cam 5.2 and Ber-EP4 in Cutaneous Squamous Cell Carcinoma.
Clarke LE, Conway AB, et al:

J Cutan Pathol 2013; 40 (July): 646-650

There are a significant number of squamous cell carcinomas (SCCs) that stain with cytokeratin 7 and CAM 5.2; thus, one should use caution when using such stains to differentiate between SCC and extramammary Paget disease.

**Background:** Cytokeratin 7 (CK7) is often seen as being a useful adjunct in staining extramammary Paget disease (EPD) while not staining cases of squamous cell carcinoma in situ. Likewise, CAM 5.2 has been touted in a similar manner. However, there have been reports of cutaneous squamous cell carcinomas (SCCs) that were CK7-and/or CAM 5.2-positive. There have also been reports that Ber-EP4 is also useful in this differential diagnosis.

**Objective:** To study the expression of CK7, CAM 5.2, and Ber-EP4 in cutaneous SCCs.

**Methods:** Cases of primary cutaneous SCC were identified, including cases of various morphologic patterns. Control cases included basal cell carcinomas (BCCs), seborrheic keratoses (SKs), and EPD. All cases were stained with CK7, CAM 5.2 and Ber-EP4.

**Results:** There were 60 cases of SCC and 10 cases from each of the controls. CK7 was present in just over one third of SCCs, including 60% of SCCs with a pagetoid pattern. CAM 5.2 demonstrated staining in 17% of SCC cases, with the majority being those SCCs with a pagetoid pattern. Ber-EP4 stained only 1 case of SCC, and the staining was weak and focal. Of the controls, all SKs were negative for all markers, the majority of BCCs stained for all 3 markers, and all cases of EPD stained for all 3 markers.

**Conclusions:** Primary cutaneous SCCs stain with CK7 and CAM 5.2, and it is noteworthy that the pagetoid variants seem to stain more commonly than the other variants of SCCs. Thus, no single marker reliably differentiates between SCC and EPD, but a panel may be of utility in such situations. Other markers studied have included CEA, GCDFP-15, EMA, and other cytokeratins. This study also found utility in Ber-EP4.

**Reviewer’s Comments:** Other tumors with pagetoid growth include melanoma, sebaceous carcinoma, and Merkel cell carcinoma. It should be emphasized that, even before considering the stains, one must carefully examine the tumor morphology and clinical scenario, as this may obviate the need for stains. (Reviewer-Stacey E. Mills, MD).

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Keywords: Cytokeratin 7, Cam 5.2, Ber-EP4, Squamous Cell Carcinoma

Print Tag: Refer to original journal article
Is There a Single-Agent, Oral Therapy for High-Risk Mantle-Cell Lymphoma?

Targeting BTK With Ibrutinib in Relapsed or Refractory Mantle-Cell Lymphoma.

Wang ML, Rule S, et al:


Single-agent, oral ibrutinib produced a 70% response rate in previously treated, relapsed or refractory mantle-cell lymphoma with comparatively much milder side effects than alternative, highly myelotoxic combination therapies.

Background: The multipart B-cell receptor (BCR) signaling pathway plays a crucial and fundamental role in B-cell antigen responses. Mutation in 1 key protein, Bruton tyrosine kinase (BTK), leads to X-linked agammaglobulinemia while other defects in the BCR pathway lead to autoimmune disease. Since many B-cell lymphomas demonstrate constitutive activation of the BCR, it is a logical target for novel drug therapy.

Objective: To investigate oral ibrutinib in patients with relapsed or refractory mantle-cell lymphoma.

Methods: In an international multicenter phase 2 study, 111 patients with relapsed or refractory mantle-cell lymphoma, some with and some without prior bortezomib therapy, received single-agent oral ibrutinib, a BTK inhibitor that irreversibly binds to its kinase domain. Academic investigators assessed overall partial or complete response rates and graded adverse events by standard methods.

Results: Among 111 relapsed or refractory mantle-cell lymphoma patients, the overall response rate was 68%, with a 47% partial response and 21% complete response rate. Both overall complete response rates improved over the course of continued oral ibrutinib therapy. After a median follow-up 15.3 months (range, 1.9 to 22.3), 46 patients continued with therapy while 65 were discontinued, 50 of whom discontinued due to disease progression. Estimated overall survival was 58% at 18 months. Thirty-eight patients (34%) demonstrated transiently increased absolute circulating lymphocytes. Most adverse events were grade 1 or 2. Less than 19% experienced decreases in neutrophils or platelets, and 6% developed pneumonia.

Conclusions: The single-agent, oral BTK inhibitor drug ibrutinib produced a 70% response rate in previously treated, relapsed or refractory mantle-cell lymphoma with comparatively milder side effects than alternative, highly myelotoxic combination therapies.

Reviewer’s Comments: The only other therapies to achieve the level of response reported in these relapsed mantle-cell lymphoma patients were intense, multidrug chemotherapy regimens (ESHAP, MINE, hyper-CVAD, and R-ICE) with much worse side effects. In a similar report, separate investigators report a similar, roughly 70% response rate in patients with relapsed or refractory chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma. Many CLL and mantle-cell lymphoma patients are reported to show transient increases in circulating lymphocytes associated with lymph node size reduction, presumably due to a block in receptor-mediated tumor cell migration to lymphoid organs. (Reviewer-Guy E. Nichols, MD, PhD).

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Keywords: B–Cell-Receptor, Lymphoma, Chronic Leukemia, Mantle-Cell, Ibrutinib

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Approaching the Biopsy for Calciphylaxis

*Cutaneous Calciphylaxis: A Retrospective Histopathologic Evaluation.*

Mochel MC, Arakaki RY, et al:

Am J Dermatopathol 2013; 35 (July): 582-586

Calcification can be difficult to find, and von Kossa and Alizarin stains are of utility as well as perieccrine and internal elastic lamina calcification.

**Background:** Calciphylaxis is relatively rare, but when it happens and you receive a case in dermatopathology, it usually is difficult to find and usually associated with a lot of phone calls. This is because calciphylaxis is a life-threatening disease that is often associated with patients in renal failure. It often occurs on the extremities and the diagnosis requires clinical pathologic correlation. Early diagnosis is especially important as prompt treatment may limit or halt disease progression. Unfortunately, while labs may show elevation in calcium or phosphorous, these studies may also be normal and may even present in patients without renal failure. The diagnostic skin biopsy demonstrates calcium within the subcutaneous vessels. Von Kossa stains may be helpful in cases. However, there are times when the biopsy is negative despite the setting of proven calciphylaxis.

**Objective:** To study the histology of calciphylaxis including the utility of von Kossa and Alizarin red special stains.

**Participants/Methods:** Cases of patients with a confirmed diagnosis of calciphylaxis were identified as well as patients who initially were suspected to have calciphylaxis but ultimately diagnosed with other disease processes. The cases were studied and also stained with von Kossa and Alizarin red special stains when possible.

**Results:** In total, there were 27 patients diagnosed with calciphylaxis, 25 of whom were patients with a history of renal failure. The other 17 patients had other diagnoses and served as a control group. While not significantly different from the control group, perieccrine and internal elastic lamina (of the muscular arteries) calcification was noted to be 100% specific for calciphylaxis. Regarding the location of the calcification, 11% of cases demonstrated perieccrine deposition, 18% of cases demonstrated internal elastic lamina calcification, 11% were located in the dermis, 29% were in the subcutaneous septae, and 16% were in the subcutaneous lobules. While von Kossa and Alizarin were comparable, the latter demonstrated larger deposits that were birefringent, and in 12% of cases, there was staining with Alizarin but lack of staining with von Kossa.

**Conclusions:** Both special stains are comparable but Alizarin staining demonstrated larger deposits that were birefringent, possibly making it easier to identify the deposits. Additionally, there were cases that stained with Alizarin but not with von Kossa. Perieccrine and internal elastic lamina calcification, while subtle, may be an additional finding to support a diagnosis of calciphylaxis.

**Reviewer's Comments:** Key points from this article are having multiple levels to evaluate and a von Kossa to also look at the perieccrine ducts and internal elastic lamina of the muscular arteries. I am not sure how common the Alizarin stain is, but perhaps tissue could be sent off for that. It is also helpful to consider additional biopsies as well. (Reviewer-William A. Kanner, MD).

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Keywords: Calciphylaxis, Von Kossa, Alizarin

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What Are Some of the Reasons for Unsatisfactory ThinPreps?

The Unsatisfactory ThinPrep® Pap Test™: Analysis of Technical Aspects, Most Common Causes, and Recommendations for Improvement.

Rosa M, Pragasam P, et al:

Diagn Cytopathol 2013; 41 (July): 588-594

Reprocessing significantly improves diagnostic yield in bloody ThinPrep specimens, but usually fails to salvage specimens with lubricant and other interferences.

Background: ThinPrep® Pap TestsTM have a number of benefits including maximal cell retrieval, homogeneous cell distribution, improved cell preparation, and reduced interference by obscuring materials. However, some institutions have reported an unexpected increase in unsatisfactory specimens following the introduction of ThinPrep testing.

Objective: To characterize the most common causes of unsatisfactory ThinPrep Pap Tests.

Design/Methods: All ThinPrep specimens collected at the pathology department at the University of Florida College of Medicine and categorized as “unsatisfactory for evaluation” from October 2010 to January 2011 were prospectively evaluated and, when appropriate, reprocessed. Reasons for the unsatisfactory result included technical problems (eg, patchy cellularity, halo effect, and thick preparations), low cellularity, and obscuring factors (eg, blood, inflammation, bacteria, gel, and mucus). Cellularity was assessed using the Bethesda 2001 criteria requiring a minimum of 5000 well-preserved, well-visualized cells and <75% obscuring material. When blood was present, reprocessing was performed based on the method recommended by Hologic, while cases with obscuring inflammation, bacteria, gel, lubricant, or mucus were reprocessed using the “dilute 1:20 method.” Cases with scant cellularity and a clean background or cytolyis were not reprocessed.

Results: 3304 cases were received during the study window, 253 (8%) of which were classified as unsatisfactory. In total, 11% (27 of 253) of the unsatisfactory cases were unsatisfactory due to scant cellularity in a clean background or cytolyis; the remaining 89% (226) of cases were all reprocessed; 40% (90) of the reprocessed cases were considered adequate after reprocessing. Unsatisfactory results were most often attributed to lubricant (42%, 96 of 226) and blood (28%, 64). Technical problems with slide preparation accounted for 15% (33) of unsatisfactory results. Scant cases with obscuring mucus, bacteria, and inflammation comprised the remaining 15% (33). When blood or technical issues were to blame, reprocessing substantially improved results, with satisfactory results obtained in 56% (39 of 64) and 91% (30 of 33), respectively. However, only 17% (16 of 96) of cases with lubricant and 24% (8 of 33) of scant cases with obscuring mucus, bacteria, and inflammation became satisfactory after reprocessing. Abnormalities (3 cases of AGC-NOS, 1 case of ASC-H, and 5 cases of ASC-US) were identified in 4% (9 of 226) reprocessed specimens.

Conclusions: Interference from lubricant and blood account for the majority of unsatisfactory ThinPrep Pap specimens. Although reprocessing frequently improves diagnostic yield for bloody specimens, it usually cannot salvage samples with lubricant and other interferences.

Reviewer’s Comments: Communication with clinical providers is critical to ensure that lubricant is not used in the collection of samples for ThinPrep Pap testing. (Reviewer-Anne McGehee Mills, MD).

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Keywords: ThinPrep Pap Test, Unsatisfactory Pap Test, Glacial Acetic Acid, Cervical Cytology, Reprocessing Cervical Cytology

Print Tag: Refer to original journal article
New Urine Test for Acute Kidney Rejection

A Rapid Noninvasive Assay for the Detection of Renal Transplant Injury.

Sigdel TK, Vitalone MJ, et al:

Transplantation 2013; 96 (July 15): 97-101

Donor-derived cell-free DNA in the urine of kidney transplant recipients is a biomarker of acute graft injury, but it is not specific for rejection.

**Background:** While tissue biopsies provide the "gold standard" means of confirming allograft rejection, noninvasive modalities have recently been proposed. In the clinical context of possible acute cardiac allograft rejection, for example, the copy number of donor-derived cell-free DNA (dd-cfDNA) in peripheral blood has been shown to correlate with acute rejection of the donor heart. In the setting of kidney transplantation, detecting dd-cfDNA in the urine would provide a non-invasive way to detect allograft injury.

**Objective:** To determine if dd-cfDNA in the urine of kidney transplant recipients is useful as a noninvasive biomarker of allograft injury.

**Methods:** 63 biopsy-matched urine samples (41 stable and 22 allograft injury) were collected and analyzed from female recipients of male donors for chromosome Y (donor)-specific dd-cfDNA. cfDNA quantification was performed using quantitative polymerase chain reaction polymerase chain reaction. A single pathologist semiquantitatively scored all matched biopsies.

**Results:** The mean urinary dd-cfDNA in acute rejection (20.5 ± 13.9) was significantly greater compared with stable grafts (2.4 ± 3.3; \( P < 0.0001 \)) or those with chronic allograft injury (2.4 ± 2.4; \( P = 0.001 \)), but no different from BK virus nephropathy (20.3 ± 15.7; \( P = 0.98 \)). In both acute rejection and BK virus nephropathy, the intrapatient drift was highly significant versus patients with stable grafts or chronic allograft injury (10.3 ± 7.4 in acute rejection; 12.3 ± 8.4 in BK virus nephropathy vs −0.5 ± 3.5 in stable grafts and 2.3 ± 2.6 in chronic allograft injury; \( P < 0.05 \)). Although most sensitive for acute allograft injury (area under the curve, 0.80; \( P < 0.0006 \); 95% confidence interval, 0.67 to 0.93), the urinary dd-cfDNA correlated with the protein/creatinine ratio \( (r=0.48; P < 0.014) \) and calculated glomerular filtration rate \( (r=-0.52; P < 0.007) \).

**Conclusions:** The level of urinary dd-cfDNA after renal transplantation appears to reflect the apoptotic injury load of the donor organ. Although serial monitoring of urinary dd-cfDNA is a sensitive biomarker of acute injury, it lacks the specificity to distinguish between acute allograft rejection and BK viral-induced injury.

**Reviewer's Comments:** The authors correctly identify the current limitations of using the drift of serum creatinine alone to provide the main indication for triggering a renal biopsy to rule out rejection. However, if the dd-cfDNA is elevated, one still cannot be sure that the cause is necessarily acute rejection. The authors clearly recognize this limitation, but offer some strong arguments in favor of noninvasive cfDNA testing rather than relying on the serum creatinine alone. (Reviewer-T. David Bourne, MD).

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Keywords: Kidney Transplantation, Biomarkers, Urinary Cell-Free DNA, Noninvasive Biomarker, Allograft Injury

Print Tag: Refer to original journal article
Multiple cystoscopic lesions and positive urine cytology are associated with recurrence and/or progression of low-grade urothelial carcinoma.

**Background:** Approximately 70% to 80% of patients with newly diagnosed bladder cancer present with nonmuscle-invasive disease. Approximately 50% to 70% of patients will recur while approximately 10% to 30% of patients will progress to muscle invasive disease. The natural biology of this process is incompletely understood with a "field change" in the normal urothelium the most accepted explanation. The diagnosis and treatment of superficial urothelial carcinoma is cystoscopy with transurethral resection while ongoing urine cytology is the most specific method for surveillance. Predicting which patients will recur/progress represents a significant clinicopathologic challenge.

**Objective:** To characterize the role of cytology and cystoscopic findings in disease recurrence and progression in patients with nonmuscle-invasive low-grade urothelial carcinoma (LGUC).

**Methods:** The authors retrieved 87 cases of nonmuscle-invasive LGUC within a 5-year period. Cystoscopic findings at the time of histologic sampling, patient demographics, and subsequent histologic and urine cytologic diagnoses were correlated using statistical analysis.

**Results:** In total, 87 patients were identified with nonmuscle-invasive LGUC including: 29 females and 58 males with a mean age of 70 years. In total, 83 of 87 cases (95.4%) demonstrated urothelial carcinoma without lamina propria invasion, 1 of 87 cases (1.1%) demonstrated lamina propria invasion, and 1 of 87 cases (1.1%) demonstrated features suspicious for lamina propria invasion. Corresponding cytology specimens obtained either at the time of biopsy or <2 months prior to biopsy demonstrated no malignant cells in 60 of 87 cases (70.0%), atypical cells in 12 of 87 (13.8%), suspicious cells in 4 of 87 cases (4.6%), and malignant cells in 9 of 87 cases (10.3%). Overall, lesions >2 cm tended toward positive/suspicious cytology. However, lesions >2 cm were not more likely to demonstrate recurrence/progression. Additionally, patients with multiple lesions tended to have abnormal (atypical or higher) urine cytology on follow-up and were more likely to have recurrence/progression of disease. Finally, patients with suspicious/positive initial cytology tended to have recurrent disease, including a trend toward the increasing likelihood of high-grade disease on subsequent histologic sampling.

**Conclusions:** The presence of multiple tumors at diagnostic cystoscopy places patients at significantly higher risk for disease recurrence, progression, and subsequent abnormal urine cytology. Additionally, lesions >2 cm tend to demonstrate positive urine cytology at diagnosis.

**Reviewer's Comments:** The current study demonstrates the importance of cystoscopic findings including size and number of tumors in assessing recurrence rates in patients with nonmuscle-invasive LGUC. The authors also recognized the value of urine cytology as a prognostic indicator and diagnostic tool in patients with nonmuscle-invasive LGUC. (Reviewer-Frank N. Moore, MD).
For small biopsies lacking definitive features of adenocarcinoma or squamous carcinoma, judicious use of one special stain each for glandular (TTF-1) or squamous (p63, CK5/6, p40) differentiation is likely to support one lineage.

**Background:** Outdated pre-2004 WHO classifications of lung tumors were based on pathologic evaluation of resection specimens. New targeted therapies and molecular testing are driving changes in practice and classification that are based primarily on pathologic evaluation of small lung biopsies and cytology samples. **Objective:** To recommend evidence-based pathology practice guidelines for handling of small lung biopsies and cytology specimens based on recent modifications to lung cancer classification and requirements for molecular testing. **Recommendations:** An unqualified diagnosis of non–small-cell carcinoma is no longer adequate since the distinction of adenocarcinoma from squamous cell carcinoma, when possible, directs special molecular testing, which in turn determines therapeutic decisions. For adenocarcinomas, algorithmic epidermal growth factor receptor mutation and ALK rearrangement testing are routine and could be extended in the future to include ROS1 testing. In the near future, squamous carcinomas may require testing for FGFR-1 amplification and/or DDR2 mutation. For large-cell carcinomas lacking definitive morphologic features of adenocarcinoma and/or squamous differentiation, judicious use of one special stain each for adenocarcinoma (TTF-1 or mucin) or squamous carcinoma (p63, CK5/6, or p40) is indicated to clarify or favor tumor lineage and preserve residual sample for molecular testing. Pathology reporting should state whether a diagnosis of adenocarcinoma, squamous carcinoma, or adenosquamous carcinoma is based on histomorphology and/or special staining. If morphology and limited special staining are not definitive, additional staining may be required to confirm carcinoma or exclude metastatic disease. A minority of cases may remain describable only as non–small-cell carcinoma. Institutional multidisciplinary practice should dictate obtaining sufficient tissue for both pathologic diagnosis and molecular testing. Residual aspiration volumes and serous effusion fluids should be preserved as paraffin cell blocks for safeguarding of potential future molecular testing. **Reviewer's Comments:** This article expands upon the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society Classification (IASLC/ATS/ERS) updated classification of adenocarcinoma in resection specimens by addressing clinically important distinctions of glandular, squamous, and neuroendocrine carcinomas in small biopsies while preserving tissue for molecular studies through sparing use of special stains. (Reviewer-Guy E. Nichols, MD, PhD).
Problematic Dermal Spindle Cell Lesion? Consider SOX10

Sox10 Expression Distinguishes Desmoplastic Melanoma From Its Histologic Mimics.
Palla B, Su A, et al:

SOX10 demonstrates strong and diffuse staining for desmoplastic melanoma, distinguishing it from most other dermal spindle cell lesions.

**Background:** There is a routine differential diagnosis for dermal spindle cell lesions of the skin, and it always includes desmoplastic melanoma. Unique to this type of melanoma is that, while it expresses S-100, other typical melanocytic markers are negative. However, S-100 staining can be nonspecific at times, complicating its interpretation. SOX10 is a marker involved in melanocyte development and has been found to be a sensitive and specific marker for melanoma.

**Objective:** To examine the utility of SOX10 staining in the differential diagnosis of desmoplastic melanoma and other dermal spindle cell lesions.

**Methods:** Cases of dermal spindle cell lesions including desmoplastic melanoma, spindle cell/poorly differentiated carcinoma, atypical fibroxanthoma, sarcoma with spindled morphology, and malignant peripheral nerve sheath tumor (MPNST) were studied. SOX10 nuclear staining was recorded.

**Results:** There were 76 tumors studied, 15 of which were desmoplastic melanomas. All cases of desmoplastic melanoma stained with SOX10, including those that were hypocellular. Thirteen of the cases demonstrated staining of >90% of tumor cells, and 2 cases demonstrated staining in 80% of tumor cells. Most cases also demonstrated 3+ intensity staining with only 2 cases demonstrating 1+ intensity. Three cases of MPNST demonstrated staining with SOX10, with a range in the staining pattern. All other cases were negative. Of note, the normal epidermal melanocytes demonstrated staining. The other normal components of the skin did not demonstrate staining.

**Conclusions:** In testing a wide variety of tumors that may be considered in the differential diagnosis with desmoplastic melanoma, SOX10 appears to be a highly specific and sensitive marker in distinguishing desmoplastic melanoma from its mimics. While MPNST also demonstrates staining, its clinical presentation is typically different, ie, that of a deep soft tissue mass. Furthermore, SOX10 staining appears “neater” as it is a nuclear stain and does not seem to stain dermal dendritic cells or histiocytes, a problem that can be seen with S-100.

**Reviewer’s Comments:** Classification of dermal spindle cell lesions can be challenging and we all want to rule in or out desmoplastic melanoma. However, only S-100 is routinely used for this and often it can lead to nonspecific staining, making interpretation difficult. Also remember that desmoplastic melanomas often have lymphoid aggregates, a helpful adjunct. However, this paper supports including SOX10 as a useful adjunct to support desmoplastic melanoma. As this may not be a marker routinely used in laboratories, it could be useful to see if your reference lab has it or can send a case of it in consultation for that purpose. (Reviewer-William A. Kanner, MD).

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**Keywords:** Desmoplastic Melanoma, SOX10, Dermal Spindle Cell Lesion

**Print Tag:** Refer to original journal article
A Multicenter Study Directly Comparing the Diagnostic Accuracy of Gene Expression Profiling and Immunohistochemistry for Primary Site Identification in Metastatic Tumors.
Handorf CR, Kulkarni A, et al:

The Pathwork Tissue of Origin Test may be of utility for poorly differentiated and undifferentiated metastatic tumors that have proven difficult to classify on immunohistochemical workup.

**Background:** Identifying the primary site for metastatic cancers often requires a combination of clinical information, imaging results, and histopathologic features. In a subset of cases, the tumors evade classification despite extensive workup. Gene expression profiling (GEP) has been proposed as a method to clarify lineage in such tumors of unknown primary, with reported accuracies ranging from 75% to 90%. However, capturing the accuracy of GEP is complicated by the fact that prospective studies on true unknown primary cases are impossible to perform, because there is no "gold standard" diagnosis.

**Objective:** To compare the diagnostic accuracy of GEP and immunohistochemistry (IHC) in assigning site-of-origin for metastatic tumors.

**Design/Methods:** Formalin-fixed paraffin-embedded specimens of 160 metastases from known primaries were prospectively collected from multiple participating institutions. In total, 33 of the cases were poorly differentiated or undifferentiated. Primary sites were required to be confirmed by clinical information and imaging and to be 1 of the 15 sites available on the GEP panel. The specimens were given masked identifiers and slides were digitized. Four blinded pathologists were tasked with evaluating the cases on the web-based interface and ordering IHC stains as they saw appropriate. After H&E evaluation and after each round of immunostaining, they recorded their diagnosis and confidence level. GEP was performed on each case using the Pathwork Tissue of Origin Test. Statistical analysis was performed to compare agreement of IHC and reference diagnosis with GEP and reference diagnosis.

**Results:** Of the 160 enrolled cases, 157 were evaluable by GEP. Overall agreement between GEP and reference diagnosis was 89.2%. This was significantly greater than the 83.3% agreement of IHC with the reference ($P = 0.013$). When analysis was restricted to 33 poorly differentiated/undifferentiated cases, GEP performed even better when compared to IHC (accuracy of 91% vs 71%; $P = 0.023$). When diagnoses could be rendered after a single round of stains, accuracy of both GEP and IHC exceeded 90%; however, when a second round of stains was required GEP exceeded IHC (83% to 67%; $P < 0.001$). Study pathologists had good-to-excellent concordance, with $\kappa$ values ranging from 0.76 to 0.83.

**Conclusions:** The Pathwork Tissue of Origin Test significantly improved on the accuracy of IHC for identifying site-of-origin for metastatic tumors, particularly in cases without obvious histologic evidence for line of differentiation and cases requiring >1 round of immunohistochemistry.

**Reviewer's Comments:** These results contrast with findings from a 2011 article (Beck et al, *Am J Surg Pathol.* 2011;35:1030-1037), which showed lackluster performance of the Tissue of Origin assay on frozen metastatic tumor specimens. An important difference between the studies is the fact that the current study limited its focus to the 15 primary locations represented on the assay panel, while the Beck study did not. (Reviewer-Anne McGehee Mills, MD).

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Keywords: Metastatic Cancer, Poorly Differentiated Cancer, Tumors of Uncertain Origin, Immunohistochemistry, Primary Site of Tumor, Gene Expression Profiling, Diagnostic Accuracy

Print Tag: Refer to original journal article
Use p40 Instead of p63 in Prostate Cancer? Not So Fast

Comparison of p40 (ΔNp63) and p63 Expression in Prostate Tissues - Which One Is the Superior Diagnostic Marker for Basal Cells?

Sailer V, Stephan C, et al:

Histopathology 2013; 63 (July): 50-56

p40 provides no advantage over p63 as a marker of basal cells in normal prostate tissues.

**Background:** The most widely used immunohistochemical stains for prostatic basal cells to aid in the diagnosis of prostatic adenocarcinoma include keratin 903 and p63. Unlike keratin 903, the p63 gene actually contains 2 promoters that result in the formation of 2 proteins, the full-length protein called TAp63, which contains an N-terminal transactivation domain, and an isoform called ΔNp63, which contains an inactive ΔN domain. Most labs use the antibody clone 4A4 that recognizes the full-length p63 protein. More recently, an antibody recognizing only the ΔN domain-containing protein, called p40, has become commercially available.

**Objective:** To determine the diagnostic value of p40 versus p63 as a basal cell marker in prostate cancer in a large patient cohort.

**Methods:** The authors retrieved 640 prostatectomy specimens in tissue microarray (TMA) format for evaluation of both malignant and normal prostatic tissues. TMA samples were submitted for immunohistochemical staining with antibodies against p63 (clone 4A4) and against p40 (rabbit polyclonal). The pattern of basal cell and secretory cell staining was evaluated semiquantitatively.

**Results:** Within normal prostatic tissue, the patterns of immunoreactivity between p63 and p40 were very similar, being identical in almost 90% of cases. Within prostatic carcinomas, however, there was additional cytoplasmic immunoreactivity seen with p40 in almost 60% of tumor cases – staining that was not seen with p63. In terms of nuclear staining, 0.6% of tumors were positive for p40 while 1.4% of tumors were positive for p63.

**Conclusions:** Nuclear staining of normal prostatic basal cells is essentially equivalent with p63 and p40. In contrast, p40 shows increased cytoplasmic staining in both normal secretory cells and in prostatic adenocarcinoma compared with p63.

**Reviewer’s Comments:** The results do not seem to provide any compelling reasons to favor p40 over p63 as a basal marker in prostate cancer. As the authors appropriately point out, the observation of increased cytoplasmic tumor cell staining with p40 would make its inclusion within an antibody cocktail containing AMACAR problematic. (Reviewer-T. David Bourne, MD).

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Keywords: Basal Cell, Immunohistochemistry, p40, p63, Prostate Cancer

Print Tag: Refer to original journal article
Rokitansky-Aschoff sinuses may demonstrate a range of benign and reactive architectural and cytologic changes.

**Background:** Rokitansky-Aschoff sinuses (RA sinuses) are invaginations of the biliary epithelium. RA sinuses may extend through and in between smooth muscle bundles and invaginate into underlying muscle layers, subserosal locations, or even perimuscular connective tissue. In these instances, benign RA sinuses may be misinterpreted as invasive carcinoma. Furthermore, sinuses may demonstrate a variety of reactive atypia secondary to underlying chronic cholecystitis with perineural and intraneural invasion described in benign RA sinuses. Ruptured RA sinuses may also demonstrate mucin extravasation associated with displaced atypical biliary glands and further complicate distinction from mucinous carcinoma.

**Objective:** To offer helpful diagnostic features to differentiate benign RA sinuses from adenocarcinoma of the gallbladder.

**Methods:** The authors retrieved 8 cases containing benign RA sinuses originally misinterpreted as adenocarcinoma. In some cases, immunohistochemical stains for p53, CEA, and Ki-67 were performed. Follow-up data were also obtained from referring pathologists, medical records, and surgical pathology reports. Eight additional cases of unambiguously benign RA sinuses were obtained for comparison.

**Results:** Clinically, 7 of 8 patients were female with a mean age of 63 years. Grossly, all cases had coexistent cholelithiasis. Histologically, the 8 cases of benign RA sinuses misinterpreted as adenocarcinoma demonstrated characteristic vertically oriented invaginations that were perpendicular to the gallbladder surface epithelium. Diagnostic confusion arose in several cases where benign RA sinuses demonstrated: (1) densely packed glands with little or even desmoplastic stroma absent marked cytologic atypia or mitotic figures; (2) irregularly branching RA sinuses absent marked cytologic atypia or mitotic figures; and (3) deeply penetrating and branching RA sinuses absent associated desmoplasmic stroma, marked cytologic atypia, or increased mitotic figures. The authors point out that desmoplastic stroma, perineural invasion, and glands present deep to the delimiting smooth muscle layers may be seen in benign RA sinuses. Immunohistochemical stains offered limited additional support with benign glands expectedly CEA–, p53–, and low Ki-67 (0% to 2%).

**Conclusions:** Invasive adenocarcinoma of the gallbladder requires both architectural and cytologic features including clear-cut invasion with desmoplasmic stroma and marked cytologic atypia with loss of polarity, large hyperchromatic or vesicular nuclei, prominent nucleoli, and mitotic figures. The reader should be aware that benign mimicry does occur.

**Reviewer's Comments:** Owing to the increasing number of cholecystectomies and existing challenges of plane of sectioning, the current article offers some practical advice on a not infrequent diagnostic dilemma. The recognition of invasive-appearing glands, perineural invasion, desmoplasmic stroma, extravasated mucin, and reactive atypia may all be seen in benign RA sinuses. As biliary carcinoma in this setting is frequently incidental, this article is a worthwhile read to remind readers of the range of benign and reactive changes possible in benign RA sinuses. (See images for this review at practicalreviews.com.) (Reviewer-Frank N. Moore, MD).

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Keywords: Rokitansky-Aschoff Sinuses, Adenocarcinoma, Reactive Atypia, CEA, p53, Ki-67

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