# Anatomic Pathology Selected Abstracts, 9/14

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## Cytokeratin 17: an adjunctive marker of invasion in anal squamous neoplastic lesions

Diagnosing anal squamous cell carcinoma, which is often preceded by anal intraepithelial neoplasia, may be challenging in small biopsies. Cytokeratin 17 (CK17) is a basal/myoepithelial cell keratin induced in activated keratinocytes and associated with disease progression in squamous cell carcinoma (SCC) of the uterine cervix, esophagus, and oral cavity. The authors investigated the utility of CK17 in diagnosing invasion in anal squamous neoplastic lesions. Immunohistochemical staining for CK17 was evaluated in 11 anal intraepithelial neoplasias (AINs), 12 invasive SCCs, eight invasive SCCs with basaloid features (BSCC), and two invasive pure basaloid carcinomas. The pattern of staining was scored as surface/central, peripheral/rim, diffuse, or absent. All cases of invasive SCC and BSCC stained positive for CK17. Eleven of 12 (92 percent) SCCs showed diffuse staining, and one of 12 (eight percent) showed peripheral staining. Six of eight (75 percent) BSCCs showed diffuse staining and two of eight (25 percent) showed peripheral staining. Both pure basaloid carcinomas were negative for CK17. One of 11 (nine percent) AINs was diffusely positive for CK17; all other AINs had surface or no CK17. Of the six patients with concurrent AIN and invasive carcinoma, superficial expression of CK17 was present in one AIN, whereas all invasive components showed diffuse staining. The sensitivity and specificity of CK17 for identifying invasion in SCC and BSCC were 100 percent and 91 percent, respectively. The authors concluded that peripheral or diffuse staining for CK17 is a useful marker of invasion in anal squamous neoplastic lesions. A potential drawback to the utility of CK17 is that the pure basaloid variant of anal carcinoma is negative for CK17.

Nazarian RM, Primiani A, Doyle LA, et al. Cytokeratin 17: an adjunctive marker of invasion in squamous neoplastic lesions of the anus. *Am J Surg Pathol.* 2014;38(1):78–85.

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## Baseline prostate inflammation relative to risk of prostate cancer in repeat biopsy

The authors conducted a study to evaluate whether baseline acute and chronic prostate inflammation among men with an initial negative biopsy for prostate cancer increased the risk of subsequent prostate cancer detection in a clinical trial with systematic biopsies. The study involved a retrospective analysis of 6,238 men aged 50 to 75 years who had prostate-specific antigen levels between 2.5 and 10 ng/mL and a prior negative biopsy in the Reduction by Dutasteride of PCa Events, or REDUCE, study and completed a two-year repeat biopsy. Prostate cancer, acute prostate inflammation, and chronic prostate inflammation were assessed by central review. The association between inflammation in baseline prostate biopsies and positive two- and four-year repeat biopsies was evaluated with the chi-square test and logistic regression analysis adjusting for baseline covariates. Acute inflammation, chronic inflammation, and a combination of both were detected in 46 baseline biopsies (one percent), 3,931 baseline biopsies (63 percent), and 892 baseline biopsies (14 percent), respectively. Acute and chronic inflammation were found to be significantly associated with each other ($P<0.001$). Acute inflammation at baseline biopsy was associated with younger age, lower prostate-specific antigen levels, and a smaller prostate (all $P<0.01$), whereas chronic inflammation was associated with older age and larger prostate glands (all $P<0.01$). At the two-year biopsy, the prevalence of prostate cancer was 14 percent (n=900 patients). On univariable and multivariable analysis, both acute and chronic inflammation were found to be significantly associated with a lower prostate

cancer risk (acute univariable: odds ratio [OR], 0.65 [P<0.001] and multivariable: OR, 0.75 [P=0.012]; and chronic univariable: OR, 0.61 [P<0.001] and multivariable: OR, 0.65 [P<0.001]). At the time of four-year biopsy, only acute inflammation was found to be associated with a lower prostate cancer risk. The authors concluded that baseline acute and chronic inflammation were independently associated with a lower prostate cancer risk. From a clinical standpoint, inflammation in negative biopsies for prostate cancer may lower the risk of subsequent prostate cancer detection.

Moreira DM, Nickel JC, Gerber L, et al. Baseline prostate inflammation is associated with a reduced risk of prostate cancer in men undergoing repeat prostate biopsy: Results from the REDUCE study. *Cancer.* 2014;120:190–196.

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## Classifying serrated lesions of the colon based on detection of BRAF V600E

BRAF V600E mutation in serrated lesions of the colon has been implicated as an important mutation and a specific marker for the serrated carcinogenic pathway. Recent findings point to microvesicular hyperplastic polyps that have similar histologic and molecular features to sessile serrated adenomas/polyps as potential colorectal precursors. The authors conducted a study to evaluate BRAF V600E mutation status by immunohistochemistry in serrated lesions of the colon with regard to histomorphology. They investigated 194 serrated lesions of the colon, comprising 42 sessile serrated adenomas/polyps, 16 traditional serrated adenomas, 136 hyperplastic polyps, and 20 tubular/tubulovillous adenomas (conventional adenomas) with the novel BRAF V600E mutation-specific antibody VE1. In addition, BRAF exon 15 and KRAS exon 2 status was investigated by capillary sequencing in selected cases. All sessile serrated adenomas/polyps (42 of 42; 100 percent), 15 of 16 (94 percent) traditional serrated adenomas, and 84 of 136 (62 percent) hyperplastic polyps were VE1+. None of the VE1- serrated lesions showed BRAF V600E mutation. Forty of 42 (95 percent) sessile serrated adenomas/polyps displayed areas with microvesicular hyperplastic polyp-like features. In microvesicular hyperplastic polyps, VE1 positivity was significantly associated with nuclear atypia (P=0.003); however, nuclear atypia was also present in VE1- cases. Immunostaining with VE1 allows not only the detection of BRAF V600E mutation but also correlation with histomorphology on a cellular level in serrated lesions. VE1 allows for the subclassification of microvesicular hyperplastic polyps according to mutation status. This improved classification of serrated lesions including immunohistochemical evaluation of BRAF V600E mutation may be the key to identifying lesions with higher potential for progressing to sessile serrated adenoma/polyp and then to BRAF V600E-mutated colorectal cancer.

Mesteri I, Bayer G, Meyer J, et al. Improved molecular classification of serrated lesions of the colon by immunohistochemical detection of BRAF V600E. *Mod Pathol.* 2014;27:135–144.

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## Molecular phenotype offoci in multifocal invasive breast carcinomas

Multiple synchronous, ipsilateral, invasive foci of breast carcinomas are frequent. Few studies have investigated the prognostic and therapeutic implications of heterogeneity of such foci. The authors reviewed the tumor type, grade, and size of all invasive foci in a series of 110 multifocal breast carcinomas documented on large-format slides. Molecular phenotype was determined by immunohistochemistry in tissue microarray blocks using three classification systems. The survival rates of patients who had tumors with microscopic (tumor type or grade, or both) heterogeneity and patients who had tumors with phenotypic heterogeneity were compared, using Kaplan-Meier curves, with the survival rates of patients who had multifocal homogeneous tumors. The hazard ratio of dying from breast cancer was also calculated. The authors found that intertumoral heterogeneity in tumor type and grade was detected in 16 of 110 tumors (14.6 percent) and six of 110 tumors (5.5 percent), respectively. The molecular phenotype of invasive tumor foci within the same breast differed in 10 to 12.7 percent of patients (11 to 14 of 110 tumors), depending on the classification system used. Patients who had phenotypically heterogeneous, multifocal cancers had a greater risk of dying from disease (hazard ratio, 2.879; 95 percent confidence interval,

1.084–7.649; P=0.034) and had significantly shorter survival rates (P=0.016). Phenotypic differences were most common in patients who had tumors that were homogeneous in terms of tumor type (11 of 18 tumors) and histology grade (14 of 18 tumors). Phenotyping additional tumor foci had the potential to influence therapeutic decisions in up to eight patients. The authors concluded that phenotyping more than one invasive focus of multifocal breast carcinomas only if the individual foci deviate microscopically appears to be insufficient because phenotypic intertumoral heterogeneity may be observed in microscopically identical foci and has potential prognostic and therapeutic consequences.

Pekar G, Gere M, Tarjan M, et al. Molecular phenotype of the foci in multifocal invasive breast carcinomas: intertumoral heterogeneity is related to shorter survival and may influence the choice of therapy. *Cancer.* 2014;120:126–134.

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## Mutation profiles of ovarian and endometrial endometrioid carcinomas

Ovarian endometrioid carcinomas and endometrial endometrioid carcinomas share many histological and molecular alterations. These similarities are likely due to a common endometrial epithelial precursor cell of origin, with most ovarian endometrioid carcinomas arising from endometriosis. To directly compare the mutation profiles of two morphologically similar tumor types—endometrial endometrioid carcinomas (n=307) and ovarian endometrioid carcinomas (n=33)—the authors performed select exon-capture sequencing on the genes ARID1A, PTEN, PIK3CA, KRAS, CTNNB1, PPP2R1A, and TP53. They found that PTEN mutations were more frequent in low-grade endometrial endometrioid carcinomas (67 percent) than in low-grade ovarian endometrioid carcinomas (17 percent). By contrast, CTNNB1 mutations were more frequent in low-grade ovarian endometrioid carcinomas (53 percent) than in low-grade endometrial endometrioid carcinomas (28 percent). This difference in CTNNB1 mutation frequency may be reflective of the distinct microenvironments—the epithelial cells lining an endometriotic cyst within the ovary are exposed to a highly oxidative environment that promotes tumorigenesis. Understanding the distinct mutation patterns found in the PI3K and Wnt pathways of ovarian and endometrial endometrioid carcinomas may provide opportunities for stratifying patients for targeted therapeutics.

McConechy MK, Ding J, Senz J, et al. Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles. *Mod Pathol.* 2014;27:128–134.

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## Link between adult-onset mastocytosis in the skin and systemic mastocytosis

Adult-onset urticaria pigmentosa/mastocytosis in the skin almost always persists throughout a person’s life. The prevalence of systemic mastocytosis in such patients is not precisely known. In this study, bone marrow biopsies from 59 patients with mastocytosis in the skin and all available skin biopsies (n=27) were subjected to a meticulous cytological, histological, immunohistochemical, and molecular analysis for the presence of World Health Organization-defined diagnostic criteria for systemic mastocytosis. Those criteria are compact mast cell infiltrates (major criterion), as well as atypical mast cell morphology, KIT D816V, abnormal expression of CD25 by mast cells, and serum tryptase levels greater than 20 ng/mL (minor criteria). Systemic mastocytosis is diagnosed when the major diagnostic criterion plus one minor criterion, or at least three minor criteria, are fulfilled. Systemic mastocytosis was confirmed in 57 (97 percent) patients by the diagnosis of compact mast cell infiltrates plus at least one minor diagnostic criterion (n=42; 71 percent) or at least three minor diagnostic criteria (n=15; 25 percent). In two patients, only two minor diagnostic criteria were detectable, which is insufficient for the diagnosis of systemic mastocytosis. Using highly sensitive molecular methods, including the analysis of microdissected mast cells, KIT D816V was found in all 58 bone marrow biopsies in which it was sought but in only 74 percent (20 of 27) of the skin biopsies. Even in cases with insufficient diagnostic criteria for systemic mastocytosis, KIT D816V-

positive mast cells were detected in bone marrow. The authors concluded that this study demonstrates that almost all patients with adult-onset mastocytosis in the skin have systemic mastocytosis with cutaneous involvement.

Berezowska S, Flaig MJ, Rueff F, et al. Adult-onset mastocytosis in the skin is highly suggestive of systemic mastocytosis. *Mod Pathol.* 2014;27:19–29.

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## Superficial CD34-positive fibroblastic tumor

Fibroblastic mesenchymal tumors show a spectrum of biological behavior ranging from benign to fully malignant. The authors reported on their two decades of experience with a distinctive, previously undescribed low-grade fibroblastic tumor of superficial soft tissues. Eighteen cases previously coded as "low-grade sarcoma, not further classified" and "malignant fibrous histiocytoma, low grade" were identified within their consultation files. The tumors occurred in adults (median age, 38 years; range, 20–76 years) of both genders (10 males and eight females), ranged in size from 1.5 to 10 cm (mean, 4.1 cm), and were confined to the superficial soft tissues of the thigh (n=5), knee (n=2), and other sites. Histological features included a fascicular growth pattern of the neoplastic spindled cells with striking, often bizarre, cellular pleomorphism and variably prominent nucleoli. Necrosis was seen in one case. All cases showed strong, diffuse CD34 positivity, and 68 percent of tested cases demonstrated focal cytokeratin expression. Desmin, EFG, FLI-1, smooth muscle actin, and S100 protein were negative. TP53 overexpression was absent. FISH studies for TGFBR3 or MGEA5 rearrangements, or both, were negative in all tested cases. Clinical follow-up was available in 13 patients (median duration, 24 months; range, 1–104 months). Twelve of 13 patients had no disease recurrence. One patient had regional lymph node metastases seven years after incomplete excision of the primary tumor. All patients were alive and disease free. The authors concluded that the unique clinicopathological features of superficial CD34-positive fibroblastic tumors define them as a novel subset of low-grade fibroblastic neoplasms, best considered to be of borderline malignancy.

Carter JM, Weiss SW, Linos K, et al. Superficial CD34-positive fibroblastic tumor: report of 18 cases of a distinctive low-grade mesenchymal neoplasm of intermediate (borderline) malignancy. *Mod Pathol.* 2014;27(2):294–302.

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## Critical appraisal of the diagnosis of sessile serrated adenoma

The sessile serrated adenoma is a relatively recently described polyp that can present diagnostic difficulties. The frequency of diagnoses varies dramatically in the reported literature. The histologic interface between the microvesicular hyperplastic polyp (MVHP) and sessile serrated adenoma (SSA) also continues to be a diagnostic problem. The trend in recent years has been toward a lower threshold for SSA diagnosis. The authors performed a cross-sectional study of 6,340 colorectal polyps received at a high-volume community-based pathology practice during a three-month period. After central review, with strict application of the diagnostic criteria outlined in the 2010 edition of the World Health Organization Classification of Tumours of the Digestive Tract, the authors found that SSAs represented 12.1 percent of all polyps. They developed novel diagnostic subcategories in an attempt to determine the most appropriate cutoff for the interface between the MVHP and SSA. The authors found that serrated polyps, whether MVHPs or SSAs, with any SSA-like crypts had clinical features more in common with the SSA than the MVHP and that this diagnostic cutoff showed good reproducibility between pathologists. This supports the position of a recent consensus publication proposing that polyps with as few as one SSA-type crypt should be diagnosed as an SSA. Applying these criteria to the authors' cohort yielded an overall SSA rate of 14.7 percent. The authors concluded that SSAs continue to be underdiagnosed in pathologic practice and that this may result in inadequate surveillance and, therefore, contribute to interval colorectal carcinomas.

Bettington M, Walker N, Rosty C, et al. Critical appraisal of the diagnosis of the sessile serrated adenoma. *Am J Surg Pathol.* 2014;38(2):158–166.

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## Intraductal papillary neoplasms of the bile duct

Intraductal papillary neoplasms of the bile duct are still poorly characterized with regard to their molecular alterations during development to invasive carcinomas, subtype stratification, and biological behavior. The authors performed a multicenter study that analyzed these issues in a large European cohort. Intraductal papillary neoplasms of the bile duct from 45 patients were graded and subtyped using mucin markers and CDX2. Tumors were also analyzed for common oncogenic pathways, and the findings were correlated with subtype and grade. Data were compared with those from 22 extra- and intrahepatic cholangiocarcinomas. Intraductal papillary neoplasms developed from preinvasive low- to high-grade intraepithelial neoplasia to invasive carcinoma. Molecular and immunohistochemical analysis revealed mutated KRAS, overexpression of TP53, and loss of p16 in low-grade intraepithelial neoplasia, whereas loss of SMAD4 was found in late phases of tumor development. Alterations of HER2, EGFR, β-catenin, and GNAS were rare events. The most common subtypes were pancreato-biliary (36 percent) and intestinal (29 percent), followed by gastric (18 percent) and oncocytic (13 percent). Patients with intraductal papillary neoplasm of the bile duct showed a slightly better overall survival rate than patients with cholangiocarcinoma (hazard ratio for cholangiocarcinoma versus intraductal papillary neoplasm of the bile duct, 1.40; 95 percent confidence interval, 0.46–4.30; P=0.552). The development of biliary intraductal papillary neoplasms of the bile duct follows an adenoma-carcinoma sequence that correlates with the stepwise activation of common oncogenic pathways. Additional large trials are needed to investigate and verify the finding of a better prognosis for intraductal papillary neoplasms compared with conventional cholangiocarcinoma.

Schlitter AM, Born D, Bettstetter M, et al. Intraductal papillary neoplasms of the bile duct: stepwise progression to carcinoma involves common molecular pathways. *Mod Pathol.* 2014;27:73–86.

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## Accuracy of estimated tumor cell percentage in molecular testing by pathologists

Molecular pathology is becoming increasingly important in present-day pathology. A major challenge of any molecular test is its ability to reliably detect mutations in samples consisting of mixtures of tumor cells and normal cells, especially when the tumor content is low. The minimum percentage of tumor cells required to detect genetic abnormalities is a major variable. Information on tumor cell percentage is essential for a correct interpretation of the result. In daily practice, the percentage of tumor cells is estimated by pathologists on H&E-stained slides, the reliability of which has been questioned. The authors conducted a study to determine the reliability of having pathologists estimate tumor cell percentages in tissue samples. A tumor area was marked on 47 H&E-stained slides of lung tumors. The percentage of tumor cells within this area was estimated independently by nine pathologists using categories of zero to five percent, six to 10 percent, 11 to 20 percent, 21 to 30 percent, and so on, until 91 to 100 percent. The percentage of tumor cells was counted manually as a gold standard. On average, the range between the lowest and highest estimate per sample was 6.3 categories. In 33 percent of estimates, the deviation from the gold standard was at least three categories. The mean absolute deviation was two categories (range between observers, 1.5–3.1 categories). A significant difference between the observers (P<0.001) was noted. If 20 percent of tumor cells were considered the lower limit to detect a mutation, samples with an insufficient tumor cell percentage (less than 20 percent) would have been estimated to contain enough tumor cells in 27 of 72 (38 percent) observations, possibly causing false-negative results. The authors concluded that estimates of tumor cell percentages on H&E-stained slides are not accurate, which could result in misinterpretation of test results. Reliability could possibly be improved by using a training set with feedback.

Smits AJ, Kummer JA, de Bruin PC, et al. The estimation of tumor cell percentage for molecular testing by pathologists is not accurate. *Mod Pathol.* 2014;27(2):168–174.

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# Radical prostatectomy or watchful waiting in early prostate cancer

Radical prostatectomy reduces mortality among men with localized prostate cancer, but important questions about long-term benefit remain. Between 1989 and 1999, the authors randomly assigned 695 men with early prostate cancer to watchful waiting or radical prostatectomy and followed them through the end of 2012 as part of the Scandinavian Prostate Cancer Group Study Number 4. The primary endpoints were death from any cause, death from prostate cancer, and risk of metastases. Secondary endpoints included initiation of androgen-deprivation therapy. During 23.2 years of follow-up, 200 of 347 men in the surgery group and 247 of 348 men in the watchful-waiting group died. Of those deaths, 63 in the surgery group and 99 in the watchful-waiting group were due to prostate cancer; the relative risk was 0.56 (95 percent confidence interval [CI], 0.41–0.77; P=0.001), and the absolute difference was 11 percentage points (95 percent CI, 4.5–17.5). The number needed to treat to prevent one death was eight. One man in the radical prostatectomy group died after surgery. Androgen-deprivation therapy was used in fewer patients who underwent prostatectomy (a difference of 25 percentage points; 95 percent CI, 17.7–32.3). The benefit of surgery with respect to death from prostate cancer was highest in men younger than 65 years of age (relative risk, 0.45) and in those with intermediate-risk prostate cancer (relative risk, 0.38). However, radical prostatectomy was associated with reduced risk of metastases among older men (relative risk, 0.68; P=0.04). The authors concluded that extended follow-up confirmed a substantial reduction in mortality after radical prostatectomy. The number needed to treat to prevent one death continued to decrease when treatment was modified according to age at diagnosis and tumor risk. A large proportion of long-term survivors in the watchful-waiting group have not required palliative treatment.

Bill-Axelson A, Holmberg L, Garmo H, et al. Radical prostatectomy or watchful waiting in early prostate cancer. *N Engl J Med.* 2014;370(10):932–942.

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