Anatomic Pathology Abstracts, 5/16

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Noninfectious aortitis of the ascending aorta: a histological and clinical correlation of cases

Aortitis is a rare but important cause of thoracic aortic disease. The authors described its histopathological patterns and associations with other aortic pathologies and systemic inflammatory disease. Database searches of thoracic specimens over 17 years and from two medical centers yielded 71 cases of noninfectious aortitis. Histological verification of tunica media inflammation was required for inclusion in the authors' evaluation. Clinical information and histopathological features were recorded. Three histological patterns emerged—necrotizing aortitis with giant cells (53), diffuse band-like aortitis (16), and "other" (two). Fifty of 53 cases of necrotizing aortitis with giant cells were isolated/idiopathic, while nine of 16 cases of diffuse aortitis had a systemic inflammatory disease. Medial degeneration (MD) was prominent in 23 of 71 cases—all in the necrotizing aortitis with giant cells category. The authors concluded that noninfectious aortitis is predominantly idiopathic/isolated in nature, occurring in elderly females. Two patterns emerged—necrotizing aortitis with giant cells, which is more likely to be idiopathic and linked to MD, suggesting a possible aetiological relationship; and diffuse aortitis, which is linked to an increased risk of systemic inflammatory disease. Therefore, knowledge of histopathological patterns may guide patient management and follow-up.

Ryan C, Barbour A, Burke L, et al. Non-infectious aortitis of the ascending aorta: a histological and clinical correlation of 71 cases including overlap with medial degeneration and atheroma—a challenge for the pathologist. *J Clin Pathol.* 2015;68:898–904.

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Stratifying HPV-induced cervical pathology using E4 with p16 or MCM

High-risk human papillomavirus types cause cervical lesions of varying severity, ranging from transient productive infections to high-grade neoplasia. Disease stratification requires examination of lesional pathology and, possibly, detection of biomarkers. P16INK4a and MCM are established surrogates of high-risk HPV E6/E7 activity and can be expressed extensively in high-grade lesions. The authors combined these two cellular biomarkers with detection of the abundant HPV-encoded E4 protein to identify productive and transforming lesions. This approach allowed the authors to distinguish true papillomavirus infections from similar pathologies and to divide the heterogeneous CIN2 category into those that are CIN1-like and express E4 and those that more closely resemble nonproductive CIN3. The authors evaluated 530 lesional areas according to standard pathology criteria and using a multiple-staining approach that allowed them to superimpose biomarker patterns, either singly or in combination, onto an annotated H&E image. Conventional grading of neoplasia was established by review panel and compared directly with the composite molecular pathology visualized on the same tissue section. The detection of E4 coincided with the onset of vacuolation, becoming abundant in koilocytes as the MCM marker declined and cells lost their defined nuclear margins as visualized by standard H&E staining. Of the dual-marker approaches, p16INK4a and E4 appeared most promising, with E4 generally identifying areas of low-grade disease even when p16INK4a was present. Extensive p16INK4a expression usually coincided with an absence of E4 expression or its focal retention in sporadic cells within the lesion. These results suggest that a straightforward molecular evaluation of HPV life-cycle deregulation in cervical neoplasia may improve disease stratification and that this can be achieved using complementary molecular biomarker pairs such as MCM/E4 or, more promisingly, p16INK4a/E4 as an adjunct to conventional pathology.

Griffin H, Soneji Y, Van Baars R, et al. Stratification of HPV-induced cervical pathology using the virally encoded molecular marker E4 in combination with p16 or MCM. *Mod Pathol.* 2015;28:977–993.

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Limited resection versus lobectomy for older patients with early-stage lung cancer

Limited resection increasingly has been used in older patients with stage 1A lung cancer. However, whether limited resection is equivalent to lobectomy according to histology is unknown. The authors identified patients older than 65 years who had stage 1A invasive adenocarcinoma or squamous cell carcinoma of 2 cm or less and were treated with limited resection (wedge or segmentectomy) or lobectomy in the Surveillance, Epidemiology, and End Results–Medicare database. They estimated propensity scores that predicted the use of limited resection and compared the survival rates of patients treated with limited resection versus lobectomy. Treatments were considered equivalent if the upper 95th percentile of the hazard ratio for limited resection was 1.25 or less. Overall, 27 percent of 2,008 patients with adenocarcinoma and 32 percent of 1,139 patients with squamous cell carcinoma underwent limited resection. Survival analyses, adjusted for propensity score using inverse probability weighting, showed that limited resection was not equivalent to lobectomy in patients with adenocarcinoma (hazard ratio [HR], 1.21; upper 95 percent confidence interval [CI], 1.34) or squamous cell carcinoma (HR, 1.21; upper 95 percent CI, 1.39). Although patients with adenocarcinoma treated with segmentectomy had survival rates equivalent to those treated with lobectomy (HR, 0.97; upper 95 percent CI, 1.07), those treated with wedge resection (HR, 1.29; upper 95 percent CI, 1.42) did not. Among patients with squamous cell carcinoma, neither wedge resection (HR, 1.34; upper 95 percent CI, 1.53) nor segmentectomy (HR, 1.19; upper 95 percent CI, 1.36) were equivalent to lobectomy. The authors concluded that limited resection generally is not equivalent to lobectomy in older patients with invasive non-small cell lung cancer of 2 cm or less, although segmentectomy may be equivalent in patients with adenocarcinoma.

Veluswamy RR, Ezer N, Mhango G, et al. Limited resection versus lobectomy for older patients with early-stage lung cancer: impact of histology. *J Clin Oncol.* 2015;33:3447–3453.

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L1CAM expression and its association with mutant p53 expression in endometrial cancer

Studies in early-stage, predominantly low- and intermediate-risk endometrial cancer have demonstrated that L1 cell adhesion molecule overexpression identifies patients at increased risk of recurrence, yet its prognostic significance in high-risk endometrial cancer is unclear. To evaluate this, as well as its frequency and the relationship of L1 cell adhesion molecule (L1CAM) with the established endometrial cancer biomarker p53, the authors analyzed the expression of both markers using immunohistochemistry in a pilot series of 116 endometrial cancers (86 endometrioid and 30 nonendometrioid subtype) with high-risk features such as high tumor grade and deep myometrial invasion. They correlated the results with clinical outcome. The authors used The Cancer Genome Atlas (TCGA) endometrial cancer series to validate their findings. Using the previously reported cutoff of 10 percent positive staining, 51 of 116 (44 percent) tumors were classified as L1CAM positive, with no significant association between L1CAM positivity and the rate of distant metastasis ($P = .195$). However, increasing the threshold for L1CAM positivity to 50 percent reduced the frequency of L1CAM-positive tumors to 24 percent (28 of 116). L1CAM expression was strongly associated with mutant p53 in the high-risk and TCGA series ($P < .001$), although a substantial fraction (36 percent of endometrioid and 10 percent of nonendometrioid morphology) of p53-mutant endometrial cancers displayed less than 10 percent L1CAM positivity. Moreover, 30 percent of p53 wild-type nonendometrioid endometrial cancers demonstrated diffuse L1CAM staining, suggesting p53-independent mechanisms of L1CAM overexpression. The authors concluded that the previously proposed threshold for L1CAM positivity of more than 10 percent does not predict prognosis in high-risk endometrial cancer, whereas an alternative threshold of greater than 50 percent does. L1CAM expression is strongly, but not universally, associated with mutant p53 and may be strong enough to serve as a prognostic marker in combination with p53. The high frequency of L1CAM expression in high-risk endometrial cancers suggests that it may also be a promising therapeutic target in this tumor subset.

Van Gool IC, Stelloo E, Nout RA, et al. Prognostic significance of L1CAM expression and its association with mutant p53 expression in high-risk endometrial cancer. *Mod Pathol.* 2016;29:174–181.

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Value of p16 staining for predicting outcome of LSIL/CIN1

The authors conducted a study to evaluate the usefulness of p16 staining in predicting the outcome of histological low-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia grade 1 (LSIL/CIN1). They prospectively recruited patients referred for colposcopy due to abnormal screening test results and diagnosed with LSIL/CIN1 at biopsy (n=507). All biopsies were stained for p16 and re-evaluated after three years by the same gynecological pathologist using the LAST criteria. Follow-up was conducted every six months and included a Pap test (liquid-based cytology), high-risk human papillomavirus testing (Hybrid Capture 2 test), and colposcopy. The mean follow-up was 28 months. An outcome diagnosis of high-grade squamous intraepithelial lesion (HSIL) was defined as a histological diagnosis of high-grade SIL/CIN (HSIL/CIN2–3). The diagnosis of LSIL/CIN1 was confirmed in 416 of 507 (82 percent) biopsies, whereas 58 (11 percent) were reclassified as negative and 33 (six percent) as HSIL/CIN2–3. During follow-up, 86 of 507 (17 percent) women initially diagnosed with LSIL/CIN1 showed an outcome diagnosis of HSIL/CIN2–3, with an HSIL final diagnosis rate of three percent (two of 58) in the women with biopsies reclassified as negative, a 17 percent (70 of 416) rate in the group with confirmed LSIL, and a 42 percent (14 of 33) rate in the women with biopsies reclassified as HSIL ($P < .001$). P16 was positive in 245 of the 507 (48 percent) patients and in 210 of 416 (50 percent) patients with confirmed LSIL/CIN1 at re-evaluation. Although positive p16 immunostaining was associated with risk of HSIL/CIN2–3 outcome in multivariate analysis (hazard ratio [HR], 1.9; 95 percent

confidence interval [CI], 1.2–3.1; $P = .009$), in the overall group of patients with LSIL/CIN1, this association was not verified in the subset of patients with confirmed LSIL/CIN1 after re-evaluation (HR, 1.6; 95 percent CI, 0.9–2.6; $P = .095$). The authors concluded that in LSIL/CIN1 lesions, p16 should be limited to equivocal cases in which HSIL/CIN2 is included in the differential diagnosis since it has low value in clinical practice as a marker of progression of LSIL/CIN1.

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Tumor budding in colorectal carcinoma: significance and histologic cutoff

Tumor budding in colorectal carcinoma has been associated with poor outcome in multiple studies, but the absence of an established histologic cutoff for high tumor budding, heterogeneity in study populations, and varying methods for assessing tumor budding have hindered widespread incorporation of this parameter in clinical reports. The authors used an established scoring system in a population-based cohort to determine a histologic cutoff for high tumor budding and to confirm its prognostic significance. They retrieved hematoxylin-and-eosin–stained sections from 553 incident colorectal carcinoma cases. Each case was previously characterized for select molecular alterations and survival data. Interobserver agreement was assessed between two gastrointestinal pathologists and a group of four general surgical pathologists. High budding (10 or more tumor buds in a 20× objective field) was present in 32 percent of cases, low budding in 46 percent, and no budding in 22 percent. High tumor budding was associated with advanced pathologic stage ($P<.001$), microsatellite stability ($P=.005$), *KRAS* mutation ($P=.010$), and, on multivariate analysis, with a greater than two times risk of cancer-specific death (hazard ratio, 2.57 [1.27, 5.19]). After multivariate adjustment by penalized smoothing splines, the authors found increasing tumor bud counts from five upward to be associated with increasingly shortened cancer-specific survival. By this method, a tumor bud count of 10 corresponded to an approximately 2.5 times risk of cancer-specific death. The interobserver agreement was good, with a weighted kappa of 0.70 for two gastrointestinal pathologists over 121 random cases and 0.72 between all six pathologists for 20 random cases. Using an established method to assess budding on routine histologic stains, the authors showed that a cutoff of 10 for high tumor budding is independently associated with a significantly worse prognosis. The reproducibility data provide support for the routine, widespread implementation of tumor budding in clinical reports.

Graham RP, Vierkant RA, Tillmans LS, et al. Tumor budding in colorectal carcinoma: confirmation of prognostic significance and histologic cutoff in a population-based cohort. *Am J Surg Pathol.* 2015;39:1340–1346.

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BRAF V600E and risk stratification of thyroid microcarcinoma

Studies from single institutions have analyzed *BRAF* in papillary microcarcinomas, sometimes with contradictory results. Most of the studies have provided limited integration of histological and clinical data. To obtain a comprehensive picture of *BRAF* V600E-mutated microcarcinomas and evaluate the role of *BRAF* testing in risk stratification, the authors performed a retrospective multicenter analysis integrating microscopical, pathological, and clinical information. The authors analyzed 365 samples from 300 patients treated at six medical institutions covering different geographical regions of Italy, with central review of all cases. *BRAF* V600E statistical analysis was conducted on 298 microcarcinomas from 264 patients after excluding those that did not meet the required criteria. *BRAF* V600E was identified in 145 of 298 (49 percent) tumors, including 35 of 37 (95 percent) tall cell ($P<.0001$) and 72 of 114 (64 percent) classic ($P<.0001$). Conversely, 94 of 129 (73 percent) follicular variant papillary microcarcinomas ($P<.0001$) were *BRAF* wild type. *BRAF* V600E-mutated microcarcinomas were characterized by markedly infiltrative contours ($P<.0001$) with elongated strings of neoplastic cells departing from the tumor and intraglandular tumor spread ($P<.0001$), typically within 5 mm of the tumor border. Multivariate

analysis correlated *BRAF* V600E with specific microscopic features (nuclear grooves, optically clear nuclei, tall cells within the tumor, and tumor fibrosis), aggressive growth pattern (infiltrative tumor border, extension into extrathyroidal tissues, and intraglandular tumor spread), higher American Thyroid Association recurrence risk group, and nonincidental tumor discovery. Showing the strongest link to *BRAF* V600E were tall cell subtype, many neoplastic cells with nuclear grooves or optically clear nuclei, infiltrative growth, intraglandular tumor spread, and a tumor discovery that was nonincidental. *BRAF* V600E-mutated microcarcinomas represent a distinct biological subtype. The mutation is associated with conventional clinicopathological features considered to be adverse prognostic factors for papillary microcarcinoma, for which it could be regarded as a surrogate marker. *BRAF* analysis may be useful to identify tumors (*BRAF* wild type) that have negligible clinical risk.

Tallini G, de Biase D, Durante C, et al. BRAF V600E and risk stratification of thyroid microcarcinoma: a multicenter pathological and clinical study. *Mod Pathol.* 2015;28:1343–1359.

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Diagnosis of Gleason pattern 4 prostatic adenocarcinoma on needle biopsy

Recognizing Gleason pattern 4 prostate carcinoma on needle biopsy is critical for patient management and prognostication. Poorly formed glands are the most common Gleason pattern 4 (GP4) subpattern. The authors studied the diagnostic reproducibility and quantitative threshold for grading GP4 poorly formed glands and the criteria to distinguish them from tangentially sectioned GP3 glands. Seventeen urologic pathologists were first queried for the definition of poorly formed glands using cases representing a spectrum of prostate carcinoma glandular differentiation. Cancer glands with no or rare lumens, elongated compressed glands, and elongated nests were considered poorly formed glands by consensus. Participants then graded a second set of 23 prostate carcinoma cases that potentially contained poorly formed glands, with fair interobserver agreement (κ=0.34). The consensus diagnoses, defined as agreement by more than 70 percent of participants, were then correlated with the quantitative (five or fewer, six to 10, and greater than 10) and topographic (clustered, immediately adjacent to, and intermixed with other well-formed prostate carcinoma glands) features of poorly formed glands in each case. Poorly formed glands immediately adjacent to other well-formed glands, regardless of their number, and small foci of five or fewer poorly formed glands, regardless of their location, were not graded as GP4. In contrast, large foci of more than 10 poorly formed glands that were not immediately adjacent to well-formed glands were graded as GP4. The authors concluded that grading poorly formed glands is challenging. However, some morphologic features, both for and against a GP4 diagnosis, are reproducible. This study represents an important step in standardizing grading of poorly formed glands based on quantitative and topographic morphologic features.

Zhou M, Li J, Cheng L, et al. Diagnosis of “poorly formed glands” Gleason pattern 4 prostatic adenocarcinoma on needle biopsy: an interobserver reproducibility study among urologic pathologists with recommendations. *Am J Surg Pathol.* 2015;39:1331–1339.

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Outcomes of a prospective active-surveillance program for favorable-risk prostate cancer

The authors conducted a study to assess the long-term outcomes for men with favorable-risk prostate cancer in a prospective, active-surveillance program. Curative intervention was recommended for disease reclassification to a higher cancer grade or volume on prostate biopsy. Primary outcomes were overall, cancer-specific, and metastasis-free survival. Secondary outcomes were the cumulative incidence of reclassification and curative

intervention. Factors associated with grade reclassification and curative intervention were evaluated in a Cox proportional hazards model. A total of 1,298 men (median age, 66 years) with a median follow-up of five years (range, 0.01–18 years) contributed 6,766 person-years of follow-up since 1995. The overall, cancer-specific, and metastasis-free survival rates were 93 percent, 99.9 percent, and 99.4 percent, respectively, at 10 years and 69 percent, 99.9 percent, and 99.4 percent, respectively, at 15 years. The cumulative incidence of grade reclassification was 26 percent at 10 years and 31 percent at 15 years. The cumulative incidence of curative intervention was 50 percent at 10 years and 57 percent at 15 years. The median treatment-free survival rate was 8.5 years (range, 0.01–18 years). Factors associated with grade reclassification were older age (hazard ratio [HR], 1.03 for each additional year; 95 percent confidence interval [CI], 1.01–1.06), PSA density (HR, 1.21 per 0.1 unit increase; 95 percent CI, 1.12–1.46), and greater number of positive biopsy cores (HR, 1.47 for each additional positive core; 95 percent CI, 1.26–1.69). Factors associated with intervention were PSA density (HR, 1.38 per 0.1 unit increase; 95 percent CI, 1.22–1.56) and a greater number of positive biopsy cores (HR, 1.35 for one additional positive core; 95 percent CI, 1.19–1.53). The authors concluded that men with favorable-risk prostate cancer should be informed of the low likelihood of harm from their diagnosis and should be encouraged to consider surveillance rather than curative intervention.

Tosoian JJ, Mamawala M, Epstein JI, et al. Intermediate and longer-term outcomes from a prospective active-surveillance program for favorable-risk prostate cancer. *J Clin Oncol.* 2015;33:3379–3385.

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Assessment of a HER2 scoring system for colorectal cancer

The authors sought to develop criteria for *ERBB2* positivity (*HER2*) in colorectal cancer to ensure accurate identification of *ERBB2*-amplified metastatic colorectal cancer patients suitable for enrollment in a phase two trial of ERBB2-targeted therapy, called the HERACLES trial. A two-step approach was used. In the first step, a consensus panel of pathologists adapted existing protocols for use in colorectal cancer to test *ERBB2* expression and amplification. Collegial revision of an archival test cohort of colorectal cancer samples led to specific recommendations for adapting current breast and gastric cancer criteria for scoring *ERBB2* in colorectal cancer. In the second step, from September 2012 to January 2015, colorectal-specific ERBB2 testing protocols and *ERBB2* scoring criteria were used to centrally screen for *ERBB2*-positive *KRAS* wild-type colorectal cancer patients to be enrolled in the HERACLES trial (a clinical validation cohort). In both archival test (N=256) and clinical validation (N=830) cohorts, a clinically sizeable five percent fraction of *KRAS* wild-type colorectal cancer patients was found to be ERBB2-positive according to the colorectal cancer-specific *ERBB2* scoring criteria. ERBB2-positive tumors showed *ERBB2* immunostaining consisting of intense membranous *ERBB2* protein expression, corresponding to homogenous ERBB2 amplification, in more than 50 percent of cells. None of the immunohistochemistry 0 or 1+ cases was amplified. Concordance between silver in situ hybridization and FISH was 100 percent. The authors propose specific criteria for defining *ERBB2* positivity in colorectal cancer (HERACLES diagnostic criteria). In a phase two trial of trastuzumab and lapatinib in a cetuximab-resistant population, HERACLES diagnostic criteria shaped the selection of patients and defined *ERBB2* as a predictive marker for response to ERBB2-targeted therapy in metastatic colorectal cancer.

Valtorta E, Martino C, Sartore-Bianchi A, et al. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. *Mod Pathol.* 2015;28:1481–1491.

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