Anatomic pathology selected abstracts

Editors: Rouzan Karabakhtsian, MD, PhD, professor of pathology and director of the Women's Health Pathology Fellowship, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY; Rachel Stewart, DO, PhD, assistant professor, Department of Pathology and Laboratory Medicine, University of Kentucky, Lexington; Nicole Panarelli, MD, associate professor of pathology, Albert Einstein College of Medicine, Montefiore Medical Center; and Shaomin Hu, MD, PhD, pathology resident, Albert Einstein College of Medicine, Montefiore Medical Center.

Diagnostic algorithmic proposal based on IHC evaluation of invasive endocervical adenocarcinomas

February 2019—The International Endocervical Adenocarcinoma Criteria and Classification was developed to separate endocervical adenocarcinomas into two main categories based on morphology: human papilloma virus-associated (HPVA) and nonhuman papilloma virus-associated adenocarcinomas. The authors aimed to improve the diagnostic accuracy of the International Endocervical Adenocarcinoma Criteria and Classification by performing a comprehensive immunohistochemical (IHC) evaluation and constructing objective IHC-based algorithms for classifying these tumors. Tissue microarrays were constructed from 297 of 409 cases used to develop the original classification. Immunostains included p16, p53, estrogen receptor, progesterone receptor, androgen receptor, vimentin, CK7, CK20, HER2, HIK1083, MUC6, CA-IX, SATB2, HNF-1beta, napsin A, PAX8, CDX2, GATA3, p63, p40, and TTF-1. High-risk human papilloma virus (HR-HPV) was detected by in situ hybridization (ISH) using probes against E6 and E7 mRNA expressed in 18 virus types. Vimentin, estrogen receptor, and progesterone receptor were expressed in a significant minority of endocervical adenocarcinomas (ECAs), primarily HPVAs, limiting their use in the differential diagnosis of endometrioid carcinoma when unaccompanied by HPV-ISH or p16. HR-HPV ISH had superior sensitivity, specificity, and negative and positive predictive values compared with p16, as published previously. HNF-1beta did not have the anticipated discriminatory power for clear cell carcinoma, nor did MUC6 or CA-IX for gastric-type carcinoma. HNF-1beta and napsin A were variably expressed in clear cell carcinoma, with HNF-1beta demonstrating less specificity as it was ubiquitously expressed in gastric-type carcinoma and the majority of HPV-associated mucinous (predominantly intestinal-type and invasive ECA resembling stratified mucin-producing intraepithelial lesion [iSMILE]) and usual-type carcinomas. HIK1083 was expressed in nearly half of gastric-type carcinomas but not in the vast majority of other subtypes. GATA3 was positive in 10 percent of usual-type adenocarcinomas and single examples of other subtypes. Rare gastric-type and HPVA mucinous carcinomas displayed HER2 overexpression. Androgen receptor was positive in six percent of usual-type adenocarcinomas. Aberrant p53 expression was found in only 3.6 percent of usual-type HPVA carcinomas, but it was more prevalent in mucinous (intestinal type and iSMILE) HPVAs and nonhuman papilloma virus associates, particularly in gastric-type carcinoma (more than 50 percent of cases). The following diagnostic classification algorithms were developed using these data. Carcinomas without overt cytoplasmic mucin (endometrioid, usual-type endocervical, clear cell, and mesonephric carcinomas) can be subclassified using HR-HPV ISH, estrogen receptor, and GATA3, whereas carcinomas with easily appreciated cytoplasmic mucin (endometrioid carcinoma with mucinous features, HPVA mucinous, and gastric-type carcinomas) can be subclassified using HR-HPV ISH and estrogen receptor.

Stolnicu S, Barsan I, Hoang L, et al. Diagnostic algorithmic proposal based on comprehensive immunohistochemical evaluation of 297 invasive endocervical adenocarcinomas. *Am J Surg Pathol.* 2018;42(8):989–1000.

Correspondence: Dr. R. A. Soslow at soslowr@mskcc.org

PD-L1 IHC staining protocols using antibody clone 28-8 on staining platforms

Several immunohistochemistry assays have been developed to assess tumor programmed death-ligand 1 expression levels in patients who are candidates for programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitor therapy. The PD-L1 IHC 28-8 pharmDx kit is FDA-approved as a complementary diagnostic and CE-marked as an in vitro diagnostic device for nivolumab therapy in melanoma and specific lung cancer subtypes, as

well as for squamous cell carcinoma of the head and neck and urothelial carcinoma in Europe. Kit availability is limited outside the United States, and its use requires the Dako Autostainer Link 48 platform, which is unavailable in many laboratories. Validated laboratory-developed tests based on 28-8 concentrated antibody outside the kit are needed. The authors conducted a study in which they compared the results from PD-L1 expression level analysis across four immunohistochemistry (IHC) platforms—Dako Autostainer Link 48, Dako Omnis, Leica Bond-III, and Ventana BenchMark Ultra—with the results from the 28-8 pharmDx kit for lung cancer (multiple histologies), melanoma, and head and neck cancer (multiple histologies). Samples were prepared per protocol for each platform and stained using the PD-L1 IHC 28-8 pharmDx kit on the Dako Autostainer Link 48 per protocol for each platform. The control samples (tonsil and placenta tissue; cell lines with prespecified PD-L1 expression levels) were tested to evaluate the specificity and sensitivity of the test assays. An agreement level of 0.90 with the pharmDx kit was set for each platform. The authors assessed inter- and intra-assay reliability. Evaluable samples were lung cancer (29), melanoma (31), and head and neck cancer (30). Mean agreement was calculated for PD-L1 expression levels of one percent or more, five percent or more, 10 percent or more, and 50 percent or more. Mean overall agreement for all indications was 0.87 to 0.99. Inter- and intra-assay of scoring/classification repeatability was 100 percent. The authors concluded that analysis of PD-L1 expression levels using laboratory-developed IHC assays with 28-8 antibody may be permissible if the platform is validated using reference samples with defined expression levels.

Koppel C, Schwellenbach H, Zielinski D, et al. Optimization and validation of PD-L1 immunohistochemistry staining protocols using the antibody clone 28-8 on different staining platforms [published online ahead of print June 26, 2018]. *Mod Pathol.* doi:10.1038/s41379-018-0071-1.

Correspondence: Dirk Zielinski at dirk.zielinski@targos-gmbh.de

Mesenteric tumor deposits as a prognostic factor in small intestinal neuroendocrine tumors

Mesenteric tumor deposits are an adverse prognostic factor for small intestinal well-differentiated neuroendocrine tumors. According to the eighth edition of the American Joint Committee on Cancer (AJCC) cancer staging manual, any mesenteric tumor deposit larger than 2 cm signifies pN2 disease. Neither this criterion nor multifocality or histologic features of mesenteric tumor deposits have been evaluated critically as prognostic factors for small intestinal neuroendocrine tumors. The authors evaluated 70 small intestinal neuroendocrine tumors with mesenteric tumor deposits for lesional contour, sclerosis, inflammation, calcification, entrapped blood vessels, and perineural invasion. They calculated Ki67 proliferative indices of the largest mesenteric tumor deposit from each case and recorded the number of tumor deposits and size of the largest deposit. The authors assessed associations between these factors—along with patient age, primary tumor Ki67 index, and AJCC stage—and the development of liver metastases and overall survival. The median mesenteric tumor deposit size was 1.5 cm (range, 0.2–7.0 cm), and the median deposit number was one (range, 1–13). Primary and tumor deposit Ki67 indices within a patient were discordant in 40 percent of cases but showed similar hazard ratios for disease-specific survival. The size of tumor deposits did not significantly affect prognosis, whether analyzed on a continuous scale or dichotomized using the recommended 2-cm cutoff. In contrast, an increasing number of deposits were associated with poor prognosis, with multiple deposits conferring an 8.19-fold risk of disease-specific death compared with a single deposit ($P = .049$). The morphologic features of the deposits had no prognostic impact. The authors concluded that the size of mesenteric tumor deposits does not affect prognosis in small intestinal neuroendocrine tumor patients, but deposit multifocality is associated with shorter disease-specific survival and should be incorporated into future staging criteria.

Gonzalez RS, Cates JMM, Shi C. Number, not size, of mesenteric tumor deposits affects prognosis of small intestinal well-differentiated neuroendocrine tumors. *Mod Pathol.* 2018;31(10):1560–1566.

Correspondence: Dr. R. S. Gonzalez at raul_gonzalez@urmc.rochester.edu

Surgical excision for flat epithelial atypia in directional vacuum-assisted biopsy of breast microcalcifications

The authors conducted a study to analyze the clinicopathological features of patients with flat epithelial atypia, diagnosed during directional vacuum-assisted biopsy targeting microcalcifications, to identify the upgrade rate to in situ ductal or invasive breast carcinoma and determine factors predicting carcinoma in the subsequent excision. They retrospectively evaluated the histological, clinical, and mammographic features of 69 cases from 65 women with directional vacuum-assisted biopsy-diagnosed flat epithelial atypia with or without atypical ductal hyperplasia or atypical lobular hyperplasia, which underwent subsequent surgical excision. Mammography was used to evaluate the extent and percentage of microcalcifications sampled by directional vacuum-assisted biopsy. All biopsy and surgical excision slides were reviewed. The women ranged in age from 40 to 85 years (mean, 57 years). All but one patient presented with only mammographically detected microcalcifications. The latter had associated architectural distortion. The extent of calcifications was less than 1 cm (n = 47), 1 to 3 cm (n = 15), more than 3 cm (n = 6), and no measurement (n = 1). A mean of 11 cores (range, 6–25) was obtained from each lesion. Post-biopsy mammogram revealed that more than 90 percent of calcifications were removed in 81 percent of cases. Pure flat epithelial atypia represented nearly two-thirds of directional vacuum-assisted biopsy specimens (n = 43; 62 percent), while flat epithelial atypia coexisted with atypical ductal hyperplasia (18 cases; 26 percent) or atypical lobular hyperplasia (eight cases; 12 percent). Upon excision, none of the cases were upgraded to in situ ductal or invasive breast cancer. In one case, however, an incidental, tubular carcinoma (4 mm) was found away from the biopsy site. Excluding this case, the upgrade rate was zero. This study adds to growing evidence that the diagnosis of flat epithelial atypia on directional vacuum-assisted biopsy for microcalcifications as the only imaging finding is not associated with a significant upgrade to carcinoma on excision. Therefore, excision may not be necessary. Furthermore, excision may not be necessary for flat epithelial atypia with atypical ductal hyperplasia limited to two terminal duct-lobular units or less if at least 90 percent of calcifications have been removed on biopsy.

McCroskey Z, Sneige N, Herman CR, et al. Flat epithelial atypia in directional vacuum-assisted biopsy of breast microcalcifications: surgical excision may not be necessary. *Mod Pathol.* 2018;31:1097–1106.

Correspondence: Dr. Nour Sneige at nsneige@houstonmethodist.org

Analysis of fibroepithelial lesions of the breast

Mammary fibroepithelial lesions encompass a wide spectrum of tumors ranging from an indolent fibroadenoma to a potentially fatal malignant phyllodes tumor. Application of the criteria used to classify them based on morphological assessment is often challenging, and there is no consensus as to what constitutes an adequate resection margin. The authors studied a retrospective cohort of 213 fibroepithelial lesions in 178 patients—80 fibroadenomas with unusual features and 133 phyllodes tumors (63 benign, 41 borderline, and 29 malignant)—to describe the spectrum of changes within each group, with special emphasis on evaluating margins. Outcome data were available for 153 fibroepithelial lesions in 139 patients (median, 56 months; range, 3–249 months). Positive final margin (tumor transected), age younger than 50 years, and a predominantly myxoid stroma were statistically significant predictors of local recurrence, while age older than 50 years, stromal overgrowth, diffuse marked atypia, necrosis, and mitotic index of 10 or more per 10 high-power fields were predictive of distant metastases. Tumors with satellite/bulging nodules were at a significantly higher risk of having a final positive resection margin. These findings highlight two important aspects of the interpretation and reporting of fibroepithelial lesions. First, the amount of myxoid stroma and the presence of satellite nodules are clinically relevant and should be routinely assessed and reported. Second, infiltrative border might not be a prerequisite for the diagnosis of malignant phyllodes tumor, while the presence of tumor necrosis, massive stromal overgrowth, or mitotic index of 25 or more per 10 high-power fields is diagnostic of malignant phyllodes tumor. On the other hand, increased mitotic index outside the range of the World Health Organization guidelines and in the absence of other worrisome features should be treated with caution since it can be found in benign tumors.

Slodkowska E, Nofech-Mozes S, Xu B, et al. Fibroepithelial lesions of the breast: a comprehensive morphological and outcome analysis of a large series. *Mod Pathol.* 2018;31:1073–1084.

Correspondence: Dr. Elzbieta Slodkowska at elzbieta.slodkowska@sunnybrook.ca

Pyloric gland adenoma of gallbladder: a unique and distinct tumor

The authors conducted a study in which they examined 24 surgically resected gallbladder pyloric gland adenomas and compared their features with the reported features of stomach, duodenum, and pancreatic pyloric gland adenomas to better understand gallbladder pyloric gland adenomas (GB-PGAs). They collected clinical information on background gallbladder lesions and histologic data, including tumor grade, existence of squamoid morules, intratumoral cholesterosis, and intracytoplasmic mucins. Immunohistochemical staining for MUC2, MUC5AC, MUC6, CDX2, pepsinogen I, p53, and MIB-1/nuclear β-catenin were evaluated. The authors also conducted targeted mutational analyses of KRAS exon 2, GNAS exon 7, and CTNNB1 exon 3. They found that 29.2 percent of the GB-PGAs were histologically high-grade dysplasias/carcinomas, 70.8 percent were low grade, and 20.8 percent and 33.3 percent contained squamoid morules and intratumoral cholesterosis, respectively. In addition, 45.8 percent and 54.2 percent of GB-PGAs were mucin-rich and mucin-poor types, respectively. Immunohistochemically, MUC6 was diffusely positive in all GB-PGAs. MUC2, MUC5AC, and CDX2 were only focally positive, and no pepsinogen I-positive cells were observed. Nuclear β-catenin accumulation was observed in all cases. However, the ratio varied among cases. Mucin-poor types were significantly associated with high histologic grade dysplasias/carcinomas and high nuclear β-catenin labeling indices. Mutational analyses identified CTNNB1 mutations in 100 percent of GB-PGAs (21 of 21), KRAS in 4.2 percent (one of 23), and GNAS in none (zero of 22). The study clarified the unique histologic features, phenotypic differentiation, and molecular statuses frequently associated with GB-PGAs. The data suggest that tumorigenesis of GB-PGA is distinct from that of stomach, duodenum, and pancreatic PGAs.

He C, Fukumura Y, Toriyama A, et al. Pyloric gland adenoma (PGA) of the gallbladder: a unique and distinct tumor from PGAs of the stomach, duodenum, and pancreas. *Am J Surg Pathol.* 2018;42(9):1237–1245.

Correspondence: Dr. Yuki Fukumura at yfuku@juntendo.ac.jp

P16 immunostaining in histological grading of anal squamous intraepithelial lesions

P16 is the most widely studied biomarker in lower anogenital tract squamous intraepithelial lesions and the only recommended biomarker for histological grade assessment. The authors conducted a systematic review and meta-analysis to evaluate p16-positivity rates according to anal squamous intraepithelial lesions/anal intraepithelial neoplasia (AIN) grade. Two investigators independently searched for studies using four electronic databases—PubMed, Web of Sciences, Scopus, and Embase—from inception until August 2017. They included studies that evaluated p16 immunostaining in histological samples of anal or perianal squamous intraepithelial lesions, or both, and that defined a p16-positive result as diffuse block staining with nuclear or nuclear plus cytoplasmic staining. A meta-analysis was performed using a random-effects model and focused on 15 studies consisting of 790 samples. The proportion of p16 expression increased with the severity of histological grade. P16 positivity was two percent (95 percent confidence interval [CI], 0.2–5 percent) in normal histology, 12 percent (95 percent CI, 2–27 percent) in low-grade squamous intraepithelial lesions (LSILs)/AIN1 (excluding condylomas), seven percent (95 percent CI, 2–13 percent) in all LSILs (AIN1/LSIL/condyloma), 76 percent (95 percent CI, 61–88 percent) in AIN2, and 90 percent (95 percent CI, 82–95 percent) in AIN3. For anal high-grade squamous intraepithelial lesions (HSILs), in studies using a two-tiered nomenclature, p16 positivity was 84 percent (95 percent CI, 66–96 percent). For all HSILs (AIN2, AIN3, HSIL combined), it was 82 percent (95 percent CI, 72–91 percent). In summary, p16 positivity in anal squamous intraepithelial lesions appears to be in a range similar to that of the commonly described cervical squamous intraepithelial lesions. However, positivity seems to be lower for anal low-grade lesions.

Albuquerque A, Rios E, Dias CC, et al. P16 immunostaining in histological grading of anal squamous intraepithelial lesions: a systematic review and meta-analysis. *Mod Pathol.* 2018;31:1026–1035.

Correspondence: Dr. Andreia Albuquerque at a.albuquerque.dias@gmail.com

Residual tumor index as a pathologic parameter in PDAC treated with neoadjuvant therapy

In the setting of neoadjuvant therapy for pancreatic ductal adenocarcinoma, accurate measurement of tumor size and, consequently, staging, based on the eighth edition of the American Joint Committee on Cancer (AJCC) cancer staging manual, is difficult. Attempts to address the limitations of tumor size in the neoadjuvant therapy (NAT) setting have included correlating residual tumor percentage with survival. However, only cases with complete pathologic response or minimal residual disease have shown better prognosis compared with all other groups. No studies have simultaneously assessed the prognostic value of tumor size and tumor regression in the setting of pancreatic ductal adenocarcinoma (PDAC) status post NAT (NAT-PDAC). Therefore, the authors conducted a study to evaluate the prognostic value of residual tumor index (RTI), a metric combining residual tumor percentage and tumor bed size as an interactional term (percentage residual tumor × tumor bed size [cm]). In a cohort of 105 cases of NAT-PDAC, they showed that RTI supersedes the prognostic value of AJCC eighth edition T staging via multivariate Cox regression. At a binary cutoff of 0.35 for RTI, the hazard ratio for recurrence-free survival was 3.26 (95 percent confidence interval, 1.51–7.04; $P < .01$). The authors further identified cutoffs of 0.2 or less, 0.2 to two, and more than two that stratified their cases into three groups via RTI, which were statistically significant in Kaplan-Meier curve analysis of recurrence-free survival ($P < .01$) and overall survival ($P < .01$). The authors concluded that RTI represents a novel metric for combining the prognostic value of tumor size and residual tumor in NAT-PDAC.

Panni RZ, Gonzalez I, Hartley CP, et al. Residual tumor index: a prognostically significant pathologic parameter in neoadjuvant-treated pancreatic ductal adenocarcinoma. *Am J Surg Pathol.* 2018;42(11):1480–1487.

Correspondence: Dr. Deyali Chatterjee at deyali@wustl.edu

Challenges in pathologic staging of renal cell carcinoma

Staging criteria for renal cell carcinoma differ from those of many other cancers because renal tumors are often spherical with subtle, finger-like extensions into veins, renal sinus, or perinephric tissue. The authors studied interobserver agreement in pathologic stage categories for challenging cases. They sent an online survey to urologic pathologists interested in kidney tumors, which yielded an 89 percent response rate (31 of 35). Most questions included one to four images, focusing on vascular and renal sinus invasion (n = 24), perinephric invasion (n = 9), and gross pathology/specimen handling (n = 17). Responses were collapsed for analysis into positive and negative/equivocal for upstaging. Consensus was considered to be agreement of 67 percent of participants. It was reached in 20 of 33 (61 percent) evaluable scenarios regarding renal sinus, perinephric, or vein invasion, of which 13 of 33 (39 percent) had 80 percent or greater consensus. Lack of agreement was encountered especially with small tumor protrusions into a possible vascular lumen, close to the tumor leading edge. Using gross photographs, most of the tumors were interpreted as suspicious but requiring histologic confirmation. Most participants (61 percent) rarely used special stains to evaluate vascular invasion, which were most commonly endothelial markers (81 percent). Most agreed that a spherical mass bulging well beyond the kidney parenchyma into the renal sinus (71 percent) or perinephric fat (90 percent) did not necessarily indicate invasion. Interobserver agreement in pathologic staging of renal cancer is relatively good among urologic pathologists interested in kidney tumors, even when selecting cases that test the earliest and borderline thresholds for extrarenal extension. However, disagreements remain, particularly for tumors with small, finger-like protrusions closely juxtaposed to the main mass.

Williamson SR, Rao P, Hes O, et al. Challenges in pathologic staging of renal cell carcinoma: a study of interobserver variability among urologic pathologists. *Am J Surg Pathol.* 2018;42(9):1253–1261.

Correspondence: Dr. Sean R. Williamson at swilli25@hfhs.org