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; Shaomin Hu, MD, PhD, staff pathologist, Cleveland Clinic; S. Emily Bachert, MD, breast pathology fellow, Brigham and Women's Hospital, Boston; and Amarpreet Bhalla, MD, assistant professor of pathology, Albert Einstein College of Medicine, Montefiore Medical Center.

A tool for technical standardization of the Ki67 immunohistochemical assay

January 2022—Ki67, a nuclear proliferation-related protein, is used extensively in anatomic pathology but has not become a companion diagnostic or a standard-of-care biomarker because of analytic variability in assay protocols and interpretation. The International Ki67 in Breast Cancer Working Group is making an effort to standardize the interpretation of Ki67. However, it has not assessed the technical issues of assay production representing multiple sources of variation, including antibody clones, antibody formats, staining platforms, and operators. The authors addressed these issues by developing a Ki67 standardization cell-line microarray system using a mixture of human

Karpas 299 or Jurkat cells ($Ki67^+$) with *Spodoptera frugiperda* 9 (Sf9; $Ki67^-$) cells in incremental ratios ranging from zero to 100 percent. To validate the tool, six different antibodies in ready-to-use or concentrate forms, from six vendors, were used to measure Ki67 proliferation indices using IHC protocols for manual (benchtop) and automated platforms. The assays were performed by three laboratories at Yale University and analyzed using QuPath and Visiopharm image-analysis software packages. The results showed statistically significant differences in Ki67 reactivity between each antibody clone. However, subsets of Ki67 assays using three clones and performed in the three labs showed no significant differences. This article details the need for analytic standardization of the Ki67 assay and highlights a new tool to address this need. The authors showed how a cell-line standardization system can be used to normalize the staining variability in proliferation indices between different antibody clones in a triple-negative breast cancer cohort. They assert that this cell-line standardization array has the potential to improve reproducibility among Ki67 assays and laboratories, which is critical for establishing Ki67 as a standard-of-care assay.

Aung TN, Acs B, Warrell J, et al. A new tool for technical standardization of the Ki67 immunohistochemical assay. *Mod Pathol*. 2021;34:1261–1270.

Correspondence: Dr. David L. Rimm at david.rimm@yale.edu

Intestinal metaplasia of the cardia: differentiating between gastric and esophageal origin

Whether intestinal metaplasia distal to the endoscopic gastroesophageal junction (that is, the cardia) is gastric or esophageal, or both, is controversial. Biopsies from this region are believed to be unreliable in resolving this issue and are not recommended. The authors set out to develop a method of histologic diagnosis for intestinal metaplasia of the cardia. They employed an expanded biopsy protocol for 986 patients, irrespective of indication for endoscopy. The biopsies sampled columnar-lined esophagus (CLE) when present, the endoscopic gastroesophageal junction defined by the proximal limit of rugal folds, the area 1 cm distal to the gastroesophageal junction, and distal stomach. The prevalence and associations of intestinal metaplasia in these four locations were evaluated. Intestinal metaplasia was found in 79 of 91 patients with CLE above the gastroesophageal junction. This was significantly associated with intestinal metaplasia at the gastroesophageal junction in 40 of 79 patients ($P<0.001$). The biopsy taken distal to the endoscopic gastroesophageal junction had intestinal metaplasia in 21 of 79 patients. No patients with CLE had intestinal metaplasia in the distal stomach. In patients without CLE, intestinal metaplasia was present at the endoscopic gastroesophageal junction or distal to it in 221 patients. In 32 patients, this was significantly associated with intestinal metaplasia in the distal stomach ($P<0.001$). The remaining 189 of 986 patients had intestinal metaplasia limited to the gastroesophageal junction

region. These data, in association with recent evidence, indicate that intestinal metaplasia limited to the area distal to the gastroesophageal junction in patients without distal gastric intestinal metaplasia represents microscopic Barrett esophagus in a dilated distal esophagus. This is mistaken for intestinal metaplasia of the proximal stomach because of a flawed endoscopic definition of the gastroesophageal junction.

Yung E, Li X, Chandrasoma P. Intestinal metaplasia of the "cardia": accurate differentiation of gastric or esophageal origin with an expanded biopsy protocol. *Am J Surg Pathol*. 2021;45(7):945–950.

Correspondence: Dr. Parakrama Chandrasoma at ptchandr@usc.edu

Grading of noninvasive low-grade papillary urothelial carcinoma with degenerative nuclear atypia

Noninvasive low-grade papillary urothelial carcinoma is a papillary neoplasm that has an orderly appearance and mild nuclear pleomorphism. Some cases show significant nuclear pleomorphism with degenerative atypia leading to grading difficulties. A retrospective review of the pathology files at the Johns Hopkins Hospital identified 16 cases diagnosed as noninvasive low-grade papillary urothelial carcinoma with degenerative atypia. Fifteen of the cases were consults. The average patient age at presentation was 46 years (range, 19–78 years). The average tumor size was 1.7 cm (range, 0.3–3.5 cm). The submitting diagnoses in consults were noninvasive high-grade papillary urothelial carcinoma (n=6), condyloma (n=1), atypical papillary lesion (n=1), prominent umbrella cells (n=1), and not given (n=6). The Ki67 proliferation rate was less than five percent in 10 of 10 cases, and the cells with large atypical nuclei were negative. There were scattered cells with nuclei at least five times the size of stromal lymphocytes but displaying smudgy chromatin and occasional multinucleation and intranuclear vacuoles. Next-generation sequencing identified the mutations *HRAS* (n=4), *FGFR3* (n=3), *KRAS* (n=3), *BRAF* (n=1), *PDGFRA* (n=1), and *PIK3CA* (n=1). Other deleterious mutations were identified, but none of them involved genes characteristic of high-grade tumors. Follow-up was available for six patients (median, 32 months). One patient recurred with a noninvasive low-grade papillary urothelial carcinoma 20 months after the index case. The remaining patients had no evidence of disease at last follow-up. No patient died or had disease progression. The combination of preservation of polarity, low mitotic activity, Ki67 of less than five percent with larger atypical nuclei negative for Ki67, and nuclear atypia that is degenerative are features used to classify these tumors as low grade.

Matoso A, Parimi V, Epstein JI. Noninvasive low-grade papillary urothelial carcinoma with degenerative nuclear atypia: a grading pitfall. *Hum Pathol*. 2021;113:1–8. doi:10.1016/j.humpath.2021.04.002

Correspondence: Dr. J. I. Epstein at jepstein@jhmi.edu

Usual interstitial pneumonia in surgical pathology: impact of consensus guidelines for idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis is a clinical syndrome characterized by usual interstitial pneumonia radiologically and pathologically. Per consensus criteria adopted in 2011, diagnosis of idiopathic pulmonary fibrosis no longer requires a biopsy in an appropriate context if usual interstitial pneumonia (UIP) is seen on imaging. Consequently, lung biopsies are typically reserved for patients who have indeterminate clinical or imaging findings or suspicion for alternative diagnoses. However, the impact of the updated guidelines on pathology practices remains unclear. The authors conducted a study to determine the frequency of histologic UIP before and after 2011. Surgical lung biopsies from adults obtained within two four-year periods: July 1, 2006 through June 30, 2010 (pre-2011 cohort) and Jan. 1, 2012 through Dec. 31, 2015 (post-2011 cohort) were studied. Two of the authors reviewed pathology slides at a multiheaded microscope in a fashion blinded to clinical information and classified them using the current guidelines. Biopsies from 177 patients before 2011 (mean age, 62 years [standard deviation, 12]; 50.3 percent [89 of 177] male) and 86 patients after 2011 (mean age, 59 years [standard deviation, 14]; 48.8 percent [42 of 86] male) were reviewed. Probable UIP or definite UIP was less frequently encountered after 2011 in all patients with fibrosis (nine of 54 [16.7 percent] versus 41 of 119 [34.5 percent] before 2011; $P=0.02$), with a

similar reduction when only patients 50 years old and older were included (eight of 46 [17.4 percent] versus 39 of 109 [35.8 percent] before 2011; $P=0.02$). There was also a concomitant rise in cases indeterminate for UIP or showing alternative diagnoses. The authors concluded that histology for UIP is less frequently encountered in their contemporary practice than in the historic era. The pretest probability of a non-UIP diagnosis is high, even in older patients, underscoring the need for pathologists to be familiar with the histologic features of alternative diagnoses.

Eldersveld JM, Yi ES, Kunze KL, et al. Usual interstitial pneumonia in contemporary surgical pathology practice: Impact of international consensus guidelines for idiopathic pulmonary fibrosis on pathologists. *Arch Pathol Lab Med*. 2021;145(6):717–727.

Correspondence: Dr. Brandon T. Larsen at larsen.brandon@mayo.edu

Predicting patterns of residual disease following neoadjuvant chemotherapy for breast cancer

The pattern of residual disease in the breast varies among breast cancer patients treated with neoadjuvant chemotherapy who do not experience a pathologic complete response. Pretreatment clinicopathologic features that predict the pattern of residual tumor are not well established. To investigate this issue, the authors performed a detailed review of histologic sections of the post-treatment surgical specimens for 665 patients with stages I through III breast cancer treated with neoadjuvant chemotherapy (NAC) followed by surgery from 2004 to 2014 and for whom slides of the post-NAC surgical specimen were available for review. The review included 242 (36.4 percent) patients with hormone receptor (HR)+/HER2- cancers, 216 (32.5 percent) with HER2+ tumors, and 207 (31.1 percent) with triple-negative breast cancer (TNBC). Slide review was blinded to pretreatment clinicopathologic features. Pathologic complete response was achieved in 7.9 percent, 37 percent, and 37.7 percent of HR+/HER2-cancers, HER2+ cancers, and TNBC, respectively ($p<0.001$). Among 389 patients with residual invasive cancer in whom the pattern of residual disease could be assessed, 287 (73.8 percent) had a scattered pattern and 102 (26.2 percent) had a circumscribed pattern. In univariate and multivariate analyses, a significant association between tumor subtype and pattern of response was found. Among patients with HR+/HER2- tumors, 89.4 percent had a scattered pattern and 10.6 percent had a circumscribed pattern. In contrast, 52.8 percent of those with TNBC had a circumscribed pattern and 47.2 percent had a scattered pattern ($p<0.001$). In addition to subtype, both histologic grade and tumor size at presentation were significantly related to the pattern of residual disease in multivariate analysis, with lower grade and larger size associated with a scattered response pattern ($p=0.002$ and $p=0.01$, respectively). A better understanding of the relationship between pretreatment clinicopathologic features of the tumor and pattern of residual disease may help guide postchemotherapy surgical management.

Pastorello RG, Laws A, Grossmith S, et al. Clinico-pathologic predictors of patterns of residual disease following neoadjuvant chemotherapy for breast cancer. *Mod Pathol*. 2021;34:875–882.

Correspondence: Dr. Stuart J. Schnitt at sschnitt@bwh.harvard.edu

Challenges of Ki67 assessment in pulmonary large-cell neuroendocrine carcinomas

The authors conducted a study to gather evidence regarding Ki67 values in large-cell neuroendocrine carcinoma and to determine whether certain cutoff values could serve as prognostic features of the disease. They used Aperio ScanScope AT Turbo, Aperio eSlide Manager, and Aperio ImageScope software (Leica Biosystems) to measure Ki67 percentages in 77 resected large-cell neuroendocrine carcinomas (LCNEC) diagnosed using World Health Organization (WHO) criteria. Overall (OS) and disease-free survival (DFS) were analyzed using the Kaplan-Meier method and SAS software (SAS Institute). Survival data were also analyzed using American Joint Committee on Cancer (AJCC) eighth edition pathological stage and six discrete Ki67 classes arbitrarily defined by 10 percent increments up to 60 percent. In addition, survival data were assessed using Ki67 classes separated by a cut-point of 20 percent or more or 40 percent or more. Tumors ranged from 0.9 to 11.5 cm, and pathological staging

comprised stages I through III. The system measured Ki67 percentage positivity using 4,072 to 44,533 tumor nuclei per case (mean, 16,610 ± 8,039). Ki67 values ranged from one to 64 percent (mean and median, 26 percent). Only 16 (21 percent) tumors had Ki67 values of 40 percent or more. Overall survival ranged from one to 298 months (median follow-up, 25 months). Disease-free survival ranged from one to 276 months (median follow-up, nine months). Overall and disease-free survival differed across AJCC stage (overall log-rank, P=0.038 and P=0.037, respectively). However, neither overall nor disease-free survival significantly correlated with stratification by Ki67 percentage into six or two classes, regardless of whether 20 percent or greater or 40 percent or greater was used as the cut-point. A literature review identified 14 papers meeting the authors' inclusion criteria by evaluating 10 or more LCNEC without comingling the results for LCNEC and small-cell carcinoma. Reported Ki67 values ranged from two to 100 percent. The authors concluded that their findings caution against a blanket use of 20 percent, 40 percent, or other Ki67 percentage cut-points for LCNEC diagnosis or prognostication.

Walts AE, Mirocha JM, Marchevsky AM. Challenges in Ki-67 assessments in pulmonary large-cell neuroendocrine carcinomas. *Histopathology*. 2021;78(5):699–709.

Correspondence: Dr. Ann E. Walts at ann.walts@cshs.org