Anatomic Pathology Abstracts, 1/17

Editors: Michael Cibull, MD, professor emeritus, University of Kentucky College of Medicine, Lexington; Rouzan Karabakhtsian, MD, PhD, associate professor of pathology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY; Thomas Cibull, MD, dermatopathologist, Evanston Hospital, NorthShore University HealthSystem, Evanston, Ill.; and Rachel Stewart, DO, resident physician, Department of Pathology and Laboratory Medicine, University of Kentucky.

Drawbacks of reflex ER and PR analysis of DCIS in breast needle core biopsies

Most institutions reflexively test all breast core needle biopsy specimens showing ductal carcinoma in situ for estrogen receptor and progesterone receptor. However, five factors suggest that this reflex testing unnecessarily increases costs. First, estrogen receptor/progesterone receptor (ER/PR) results do not impact the next step in standard therapy—namely, surgical excision. Second, a subset of surgical excisions performed for ductal carcinoma in situ (DCIS) diagnosed on core needle biopsy will harbor infiltrating mammary carcinoma, which will then need to be retested for ER/PR. Third, because ER and PR labeling is often heterogeneous in DCIS, negative ER/PR results on small core needle biopsy specimens should be repeated on surgical excision specimens with larger amounts of DCIS to confirm that the result is negative. Fourth, many patients with pure ER/PR-positive DCIS after surgical excision will decline hormone therapy, rendering ER/PR testing unnecessary. Fifth, PR status in DCIS has no proven independent value. The authors examined the unnecessary added costs associated with reflex ER/PR testing of DCIS on core needle biopsy specimens due to these factors. They reviewed 58 core needle biopsies showing pure DCIS that also had a resulting surgical excision specimen. The review was conducted at the authors' institution over a two-year period. No patients received neoadjuvant hormone therapy. On surgical excision, five (8.6 percent) cases had only benign findings, 44 (75.9 percent) had pure DCIS, and nine (15.5 percent) had DCIS with invasive mammary carcinoma. The nine cases with invasive mammary carcinoma in the surgical excision specimen and four pure DCIS in surgical excision specimens that were ER/PR negative on core needle biopsy would need to repeat ER/PR testing. The total unnecessary increased cost for core needle biopsy specimen testing of these 13 cases was $8,148.92 (approximately $140 per patient for the 58 patients in the study). The authors found that ER/PR testing results impacted patient management in only 16 of 49 (33 percent) pure DCIS cases after surgical excision, indicating that $20,685.72 (approximately $357 per patient in the study) had been spent for unnecessary ER/PR testing. PR testing could have been omitted in the 16 cases in which ER/PR results were used, which would have saved $5,014.72, or $86.46 per patient. Extrapolating the increased cost of $583 per DCIS diagnosis on core needle biopsy to 60,000 new cases of DCIS in the United States each year, reflex core needle biopsy ER/PR testing would unnecessarily increase costs by approximately $35 million. The authors discourage the practice of reflexively ordering ER/PR on core needle biopsy specimens or surgical excision

specimens containing DCIS. Instead, they recommend that ER alone be performed on surgical excision specimens only when hormone therapy is a serious consideration after medical oncology consultation.

VandenBussche CJ, Cimino-Mathews A, Park BH, et al. Reflex estrogen receptor (ER) and progesterone receptor (PR) analysis of ductal carcinoma in situ (DCIS) in breast needle core biopsy specimens: an unnecessary exercise that costs the United States $35 million/y. Am J Surg Pathol. 2016;40:1090–1099.

Correspondence information not provided.

[hr]

Analysis of eosinophils and mast cells of gastrointestinal tract in healthy children

Many gastrointestinal disorders, including GI eosinophilia and inflammatory bowel disease, can be characterized by increased mucosal eosinophils or mast cells. The authors assessed the mucosal cellular counts along the GI tract of healthy children in a Canadian pediatric population to establish a benchmark reference. They quantified the eosinophils and mast cells from 356 mucosal biopsies of the GI tract obtained during upper and lower endoscopic biopsies of 38 patients in eastern Ontario. The mean total counts of eosinophils varied for the 11 tissues examined—from a low of 7.6 ± 6.5/high-power field (HPF) (×40[×400, 0.55 mm2]) in the body of the stomach to a high of 50.3 ± 17.4/HPF in the cecum. The lower GI tract, which comprises the ileum, cecum, colon, sigmoid, and rectum, generally had higher total eosinophil counts than the upper GI tract, which comprises the antrum and body of the stomach, duodenum, and duodenal cap (combined average, 32.1 ± 20.6 versus 19.3 ± 15.8, respectively). Similarly, the number of mucosal mast cells in the GI tract varied by region—ranging from 0.04 ± 0.2/HPF in the duodenal cap to 0.9 ± 2.6/HPF in the ileum. The total counts for eosinophils and mast cells in the lamina propria were not significantly different based on gender when adjusted for multiple testing. Eosinophil polarity was absent in many cases, irrespective of the GI region. The authors concluded that the numeration and localization of eosinophils and mast cells will provide normative data for upper and lower endoscopic GI biopsies in the pediatric population of eastern Ontario.

Chernetsova E, Sullivan K, de Nanassy J, et al. Histologic analysis of eosinophils and mast cells of the gastrointestinal tract in healthy Canadian children. *Hum Pathol.* 2016;54:55–63.

Correspondence: Dr. Dina El Demellawy, 401 Smyth Road, Ottawa, ON, Canada, K1H 8L1

[hr]

Classifying gastric cancer into molecular subgroups

The overall survival of gastric carcinoma patients remains poor despite improved control of risk factors and surveillance. This highlights the need for new classifications driven toward identifying potential therapeutic targets. Using sophisticated molecular technologies and analysis, three groups recently provided genetic and epigenetic molecular classifications of gastric cancer—The Cancer Genome Atlas, “Singapore-Duke” study, and Asian Cancer Research Group. Taking into account these classifications, the authors examined the expression of 14 biomarkers in a cohort of 146 gastric adenocarcinomas and performed unsupervised hierarchical clustering analysis using less expensive and widely available immunohistochemistry and in situ hybridization techniques. They identified five groups of gastric cancers, which were based on Epstein-Barr virus positivity, microsatellite instability, aberrant E-cadherin, and p53 expression; the remaining cases constituted a group characterized by normal p53 expression. The five categories corresponded to the reported molecular subgroups by virtue of clinicopathologic features. Evaluation of these clusters and survival using the Cox proportional hazards model showed a trend for superior survival in the Epstein-Barr virus and microsatellite-unstable related adenocarcinomas. Based on their findings, the authors propose a simplified algorithm that is able to reproduce the recently proposed molecular subgroups of gastric adenocarcinoma using immunohistochemical and in situ hybridization techniques.

Setia N, Agoston AT, Han HS, et al. A protein and mRNA expression-based classification of gastric cancer. *Mod Pathol.* 2016;29:772–784.

Correspondence: Dr. G. Y. Lauwers at glauwers@partners.org

[hr]

Clinicopathologic significance of mismatch repair defects in endometrial cancer

The clinicopathologic significance of mismatch repair defects in endometrioid endometrial cancer has not been definitively established. The authors performed tumor typing to classify mismatch repair (MMR) defects to determine if MMR status is prognostic or predictive. They assessed primary endometrioid endometrial cancers from NRG/GOG0210 patients for microsatellite instability, *MLH1* methylation, and MMR protein expression. Each tumor was assigned to one of four MMR classes: normal, epigenetic defect, probable mutation (MMR defect not attributable to *MLH1* methylation), or microsatellite instability-low. The relationships between MMR classes and clinicopathologic variables were assessed using contingency table tests and Cox proportional hazards models. A total of 1,024 tumors were assigned to MMR classes. Epigenetic and probable mutations in MMR were significantly associated with higher grade and more frequent lymphovascular space invasion. Epigenetic defects were more common in patients with higher International Federation of Gynecology and Obstetrics stage. Overall, no differences in outcomes were found. However, progression-free survival was worse for women whose tumors had epigenetic MMR defects compared with the MMR normal group (hazard ratio, 1.37; $P<.05$; 95 percent confidence interval, 1.00–1.86). An exploratory analysis of interaction between MMR status and adjuvant therapy showed a trend toward improved progression-free survival for probable MMR mutation cases. The authors concluded that MMR defects in endometrioid endometrial cancers are associated with a number of well-established poor prognostic indicators. Women with tumors that had MMR defects were likely to have higher-grade cancers and more frequent lymphovascular space invasion. Surprisingly, the outcomes in these patients were similar to those of patients with MMR normal tumors, suggesting that MMR defects may counteract the effects of negative prognostic factors.

McMeekin DS, Tritchler DL, Cohn DE, et al. Clinicopathologic significance of mismatch repair defects in endometrial cancer: An NRG Oncology/Gynecologic Oncology Group Study. *J Clin Oncol.* 2016;34:3062–3068.

Correspondence: Dr. Paul J. Goodfellow at paul.goodfellow@osumc.edu

[hr]

Use of immunostains to distinguish hepatic adenoma from hepatocellular carcinoma

Immunostains are used to subtype hepatic adenomas to stratify for the risk of malignant transformation. The most common panel of immunostains used for this purpose includes liver fatty acid-binding protein (LFABP), serum amyloid A protein, C-reactive protein, and glutamine synthetase. Some pathologists use these stains in an attempt to distinguish hepatocellular carcinomas from hepatic adenomas. However, data on the performance of these stains in hepatocellular carcinoma are limited. To investigate the staining characteristics of hepatocellular carcinomas, the authors studied 159 such cases and seven fibrolamellar carcinomas (92 well differentiated, 67 moderately differentiated, and seven poorly differentiated) for the expression of LFABP, serum amyloid A, C-reactive protein, and glutamine synthetase. All of the stains were positive in at least one subset of hepatocellular carcinoma: serum amyloid A was positive in 27 of 159 (17 percent) cases, C-reactive protein in 86 of 159 (54 percent), and glutamine synthetase in 23 of 47 (49 percent), while LFABP showed loss of staining in 36 of 159 (23 percent) cases. Fibrolamellar carcinomas were consistently C-reactive protein positive (seven of seven cases) and frequently showed loss of LFABP (four of seven cases). No association was found between expression of serum amyloid A, C-reactive protein, and glutamine synthetase, as well as loss of LFABP expression and other

clinicopathologic features. Hepatocellular carcinomas with loss of LFABP were more frequently associated with negative glutamine synthetase expression (11 of 14 cases; *P*=.02). The authors concluded that these data show that immunostains used to subtype hepatic adenomas are not useful for distinguishing hepatocellular carcinomas from hepatic adenomas and should be used only after a diagnosis of hepatic adenoma has been made using other criteria.

Liu L, Shah SS, Naini BV, et al. Immunostains used to subtype hepatic adenomas do not distinguish hepatic adenomas from hepatocellular carcinomas. *Am J Surg Pathol.* 2016;40:1062–1069.

Correspondence information not provided.

[hr]

Prognostic effect of PD-L1 expression patterns in cervical cancers

Programmed death-ligand 1 (PD-L1) is expressed in various immune cells and tumor cells and can bind to programmed death 1 (PD-1) on T lymphocytes, thereby inhibiting their function. The PD-1/PD-L1 axis is a major immunotherapeutic target for checkpoint inhibition in various cancer types, yet information about the clinical significance of PD-L1 expression in cervical cancer is largely lacking. The authors studied PD-L1 expression in paraffin-embedded samples from two cohorts of patients with cervical cancer: primary tumor samples from cohort one (squamous cell carcinoma, n=156; adenocarcinoma, n=49) and primary and paired metastatic tumor samples from cohort two (squamous cell carcinoma, n=96; adenocarcinoma, n=31). Squamous cell carcinomas were more frequently positive for PD-L1 and also contained more PD-L1–positive tumor-associated macrophages than did adenocarcinomas (both *P*<.001). PD-L1–positive tumor-associated macrophages were found to express CD163 or CD14, or both, by triple fluorescent immunohistochemistry, demonstrating an M2-like phenotype. Disease-free survival (*P*=.022) and disease-specific survival (*P*=.046) were significantly worse in squamous cell carcinoma patients who had diffuse PD-L1 expression than in patients who had marginal PD-L1 expression in primary tumors. Disease-specific survival was significantly worse in adenocarcinoma patients with PD-L1–positive tumor-associated macrophages than in adenocarcinoma patients without PD-L1–positive tumor-associated macrophages (*P*=.014). No differences in PD-L1 expression between primary tumors and paired metastatic lymph nodes were detected. However, PD-L1–positive immune cells were found in greater abundance around the metastatic tumors than the paired primary tumors (*P*=.001 for squamous cell carcinoma and P=.041 for adenocarcinoma). These findings point to the key role of PD-L1 in immune escape for cervical cancer and provide a rationale for therapeutic targeting of the PD-1/PD-L1 pathway.

Heeren AM, Punt S, Bleeker MC, et al. Prognostic effect of different PD-L1 expression patterns in squamous cell carcinoma and adenocarcinoma of the cervix. *Mod Pathol.* 2016;29:753–763.

Correspondence: Dr. E. S. Jordanova at e.jordanova@vumc.nl

[hr]

Variation in pattern-based classification of invasive endocervical adenocarcinoma

A pattern-based classification for invasive endocervical adenocarcinoma has been proposed as predictive of the risk of nodal metastases. The authors aimed to determine the reproducibility of such a classification in the context of common diagnostic challenges: distinguishing between in situ and invasive adenocarcinoma and measuring depth of invasion. Nine gynecologic pathologists independently reviewed 96 cases of endocervical adenocarcinoma (two slides per case). They diagnosed each case as in situ or invasive carcinoma, classifying the latter following the pattern-based classification as pattern A (nondestructive), B (focally destructive), or C (diffusely destructive). Depth of invasion, when applicable, was measured (mm). Overall, diagnostic reproducibility of pattern diagnosis

was good (κ = 0.65). Perfect agreement (nine of nine reviewers) was seen in only 11 cases (11 percent), all destructively invasive (10 pattern C and one pattern B). In all, concordance between five or more of the nine reviewers was achieved in 82 of 96 (85 percent) cases. Distinction between in situ and invasive carcinoma, regardless of pattern, showed only slight agreement (κ = 0.37). Likewise, distinction restricted to in situ versus pattern A was poor (κ = 0.23). Distinction between nondestructive (in situ plus pattern A) and destructive (patterns B plus C) carcinoma showed significantly higher agreement (κ = 0.62). Estimation of depth of invasion showed excellent reproducibility (intraclass correlation coefficient, 0.82). However, different measurements resulting in differing FIGO stages were common (from at least one reviewer in 79 percent of cases). On the basis of interobserver agreement, the pattern-based classification is best at diagnosing destructive invasion, which carries a risk for nodal metastases. Agreement in diagnosing in situ versus invasive carcinoma, including pattern A, was poor. Given the nil risk of nodal spread in in situ and pattern A lesions, the term endocervical adenocarcinoma with nondestructive growth can be considered when the distinction is difficult and after excluding destructive invasion. Because the depth of invasion measurement was highly reproducible among pathologists, the pattern-based approach can complement, but should not replace, the depth of invasion metric.

Parra-Herran C, Taljaard M, Djordjevic B, et al. Pattern-based classification of invasive endocervical adenocarcinoma, depth of invasion measurement and distinction from adenocarcinoma in situ: interobserver variation among gynecologic pathologists. *Mod Pathol.* 2016;29:879–892.

Correspondence: Dr. C. Parra-Herran at carlos.parra.herran@utoronto.ca

[hr]