Anatomic Pathology Abstracts, 7/17

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.

Potential quality indicators for lymph node staging of colon cancer

Evaluation of 12 or more lymph nodes is used as a quality indicator for adequacy of pathologic examination of colon cancer resections. The authors conducted a study to evaluate the utility of a focused lymph node search in the immediate vicinity of the tumor and a "second-look" protocol for improving lymph node staging in colon cancer. Lymph nodes were submitted separately from the primary nodal basin (PNB) and secondary nodal basin (SNB), defined as an area less than 5 cm away and an area greater than 5 cm away from the tumor edge, respectively, in 201 consecutive resections from 2010 to 2013. One hundred sixty-eight consecutive tumors (2006–2009) were used as a control group. A second search was performed in all cases that were N0 after the first search. In those cases, 20.9 ± 10.8 lymph nodes were collected from the PNB, compared with 8.5 ± 9.1 from the SNB. Positive lymph nodes were found in N+ tumors in the PNB in all cases but in only nine percent (four of 46) of SNBs ($P < .001$). A second search increased the lymph node count by an average of 10 nodes. In five of 114 (4.4 percent) cases, N0 after the first search converted to N+ after a second search that yielded one to four positive lymph nodes, all of which were in the PNB. The authors concluded that emphasis on the number of lymph nodes examined from the PNB and a second-look protocol improve nodal staging.

Lisovsky M, Schutz SN, Drage MG, et al. Number of lymph nodes in primary nodal basin and a "second look" protocol as quality indicators for optimal nodal staging of colon cancer. *Arch Pathol Lab Med.* 2017;141:125–130.

Correspondence: Dr. Amitabh Srivastava at asrivastava@partners.org

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A study addressing diagnostic concordance of breast pathologists

Previous concordance studies examining the accuracy of breast diagnosis by pathologists—typically targeting difficult, histologically challenging breast lesions using artificial and restrictive environments—have reported aberrantly high levels of diagnostic discordance. The results of these studies may be misinterpreted by nonpathologists and raise concerns relating to routine practice. The authors conducted a study to assess the diagnostic agreement among breast pathologists in the United Kingdom. They reviewed 240 consecutive breast

lesions (submitted by participants from their routine practice) included in the UK National Health Service Breast Screening Programme breast pathology external quality assurance scheme during the past 10 years. An average of approximately 600 participants viewed each case. Data on diagnostic categories—benign, atypical, in situ malignant, and invasive malignant—were collected. The study grouped together benign and atypical diagnoses. The overall diagnostic agreement level was in the almost perfect range. Thirty-five (14.6 percent) cases showed diagnostic concordance of 95 percent or less. The reasons for discordance included scheme methodology limitations such as miscoding of certain lesions—for example, phyllodes tumors and lobular neoplasia (n = 7)—and variable representation of the index lesion on glass slides (n = 18), as well as the inclusion of diagnostically challenging cases that may be interpreted more easily using immunohistochemistry (n = 28). The latter included benign and malignant papillary lesions (n = 12), complex sclerosing lesions (n = 7), intraductal epithelial proliferative lesions (n = 6), and an unusual special tumor type (n = 1). Further review identified pathologist misinterpretation in 13 (5.4 percent) cases, with an average discordance rate of only 4.2 percent. The authors concluded that the performance of breast pathologists is high. Exclusion of the effect of the scheme methodology limitations further highlights the high performance rate and identifies true diagnostically challenging entities. These difficult cases may benefit from additional diagnostic workup and second opinions.

Rakha EA, Ahmed MA, Aleskandarany MA, et al. Diagnostic concordance of breast pathologists: lessons from the National Health Service Breast Screening Programme Pathology External Quality Assurance Scheme. *Histopathol.* 2017;70:632–642.

Correspondence: Emad A. Rakha at emadrakha@yahoo.com or emad.rakha@nuh.nhs.uk

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PD-L1 expression and response to pembrolizumab in melanoma

Expression of programmed death-ligand 1 is a potential predictive marker for response and outcome after treatment with anti-programmed death 1. The authors explored the relationship between anti-programmed death 1 activity and programmed death-ligand 1 (PD-L1) expression in patients with advanced melanoma who were treated with pembrolizumab in the phase Ib KEYNOTE-001 study (clinical trial information: NCT01295827). In the study, 655 patients received 10 mg/kg of pembrolizumab once every two weeks or three weeks or 2 mg/kg once every three weeks. Tumor response was assessed every 12 weeks per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 by independent central review. The primary outcome was objective response rate. Secondary outcomes included progression-free survival and overall survival. Membranous PD-L1 expression in tumor and tumor-associated immune cells was assessed by a clinical trial immunohistochemistry assay (22C3 antibody) and scored on a unique melanoma scale of zero to five by one of three pathologists who were blinded to clinical outcome. A score of two or more (membranous staining in one percent or more of cells) was considered positive. Of 451 patients with evaluable PD-L1 expression, 344 (76 percent) had PD-L1–positive tumors. Demographic and staging variables were equally distributed among PD-L1–positive and –negative patients. An association between higher melanoma score and higher response rate and longer progression-free survival (hazard ratio, 0.76; 95 percent confidence interval [CI], 0.71–0.82) and overall survival (hazard ratio, 0.76; 95 percent CI, 0.69–0.83) was observed ($P < .001$ for each). The objective response rate was eight, 12, 23, 43, 57, and 53 percent for melanoma zero, one, two, three, four, and five, respectively. The authors concluded that PD-L1 expression in pretreatment tumor biopsy samples was correlated with response rate, progression-free survival, and overall survival. However, patients with PD-L1–negative tumors may also achieve durable responses.

Daud AI, Wolchok JD, Robert C, et al. Programmed death-ligand 1 expression and response to the anti-programmed death 1 antibody pembrolizumab in melanoma. *J Clin Oncol.* 2016;34:4102–4109.

Correspondence: Adil I. Daud at adaud@medicine.ucsf.edu

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P16ink4 and CK7 immunostaining to predict HSIL outcome for LSIL

P16ink4 and cytokeratin 7 have been proposed to identify low-grade squamous intraepithelial lesions (LSIL) at greater or lesser risk for an outcome of high-grade squamous intraepithelial lesion (HSIL). The authors correlated cytokeratin 7 (CK7) and p16ink4 staining in LSILs with outcome on follow-up and placed this information in the context of prior reports. Cervical LSIL biopsies with at least one year of follow-up information were immunostained for CK7 and p16ink4. Follow-up outcomes included no SIL, LSIL (persistence), or HSIL (CIN2+). A total of 109 LSILs were studied, and 18.3 percent stained positive for CK7. Ninety-one percent of CK7-negative LSILs regressed, 4.5 percent persisted, and 4.5 percent had an HSIL outcome versus 60, 20, and 20 percent of CK7-positive LSILs, respectively ($P=0.036$). P16ink4 status was not significantly associated with outcome. Review of the literature revealed a highly variable rate of positive p16ink4 immunoreactivity in LSIL and CIN2+ outcome for p16-positive LSIL but a consistently high negative predictive value (more than 90 percent) in the case of no or low p16 expression. Interobserver reproducibility for the diagnosis of CIN2 in the literature ranged from poor to good, with unanimous agreement on the diagnosis of CIN2 occurring in fewer than 25 percent of cases. As with high-risk human papillomavirus testing, the most clinically useful result of p16ink4 staining is a negative test, implying no lesion or CIN1 and conferring a low risk of HSIL outcome. HSIL outcomes (progression) are highly variable and are subject to wide differences in interobserver interpretation for CIN2. This argues against relying on p16ink4 to predict CIN2+ or to upgrade CIN1 to CIN2. It also begs the question of whether CIN2 should be replaced by an alternate and less pejorative term (SIL of intermediate grade) for lesions that are not reproducibly classified as LSIL or HSIL, with an appropriate management scheme.

Huang EC, Tomic MM, Hanamornroongruang S, et al. p16ink4 and cytokeratin 7 immunostaining in predicting HSIL outcome for low-grade squamous intraepithelial lesions: a case series, literature review and commentary. *Mod Pathol.* 2016;29:1501–1510.

Correspondence: Dr. E. C. Huang at ecchuang@ucdavis.edu

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Diagnosis of T1 colorectal cancer in pedunculated polyps in clinical practice

T1 colorectal cancer can be mimicked by pseudoinvasion in pedunculated polyps. British guidelines are one of the few that recommend diagnostic confirmation of T1 colorectal cancer by a second pathologist. The authors conducted a study to provide insights into the accuracy of the histological diagnosis of pedunculated T1 colorectal cancer in daily clinical practice. They selected for histologic review a sample of 128 cases, from 10 Dutch hospitals, diagnosed as pedunculated T1 colorectal cancer between 2000 and 2014. First, two Dutch expert gastrointestinal pathologists reviewed all H&E-stained slides. In 20 cases, the diagnosis of T1 colorectal cancer was not confirmed (20 of 128; 16 percent). The discordant cases were subsequently discussed with a third Dutch gastrointestinal pathologist and a consensus diagnosis was reached. The revised diagnoses were pseudoinvasion in 10 cases (10 of 128; eight percent), high-grade dysplasia in four cases (four of 128; three percent), and equivocal in six cases (six of 128; five percent). To further validate the consensus diagnosis, the discordant cases were reviewed by an independent expert pathologist from the United Kingdom. A total of 39 cases were reviewed blindly, including the 20 cases with a revised diagnosis and 19 control cases where the Dutch expert panel agreed with the original reporting pathologist’s diagnosis. In 19 of the 20 cases with a revised diagnosis, the British pathologist agreed that T1 colorectal cancer could not be confirmed. Furthermore, among the 19 control cases, the British pathologist was unable to confirm T1 colorectal cancer in an additional four cases and was equivocal in three cases. The authors concluded that both generalist and expert pathologists experience diagnostic difficulty distinguishing pseudoinvasion and high-grade dysplasia from T1 colorectal cancer. To prevent overtreatment, review of the histology of pedunculated T1 colorectal cancers by a second pathologist should be considered, with discussion of these cases at a multidisciplinary meeting.

Backes Y, Moons L, Novelli MR, et al. Diagnosis of T1 colorectal cancer in pedunculated polyps in daily clinical

practice: a multicenter study. *Mod Pathol.* 2016;30:104–112.

Correspondence: Dr. M. M. Lacle at m.m.lacle@umcutrecht.nl

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Molecular genetic heterogeneity in undifferentiated endometrial carcinomas

Undifferentiated and dedifferentiated endometrial carcinomas are rare and highly aggressive subtypes of uterine cancer that are not well characterized at the molecular level. The authors conducted a study to determine whether dedifferentiated carcinomas carry molecular genetic alterations similar to those of pure undifferentiated carcinomas and to gain insight into the pathogenesis of these tumors. They selected a cohort of 18 undifferentiated endometrial carcinomas, eight of which had a well-differentiated endometrioid carcinoma component (dedifferentiated endometrioid carcinomas), and studied them using immunohistochemistry and massive parallel and Sanger sequencing. They also carried out whole exome sequencing of the endometrioid and undifferentiated components, as well as normal myometrium, in one case. According to the Cancer Genome Atlas classification, the authors distributed 95 percent of the undifferentiated carcinomas in this series as hypermutated tumors with loss of mismatch repair protein expression and microsatellite instability (eight cases; 45 percent); ultramutated carcinomas carrying mutations in the exonuclease domain of *POLE* (two cases; 11 percent); high copy-number alterations (copy-number high) tumors exhibiting only *TP53* mutations with a high number of alterations detected by FISH (two cases; 11 percent); and low copy-number alterations (copy-number low) tumors with molecular alterations typical of endometrioid endometrial carcinomas (five cases; 28 percent). However, two of the latter cases also had *TP53* mutations and a higher number of alterations detected by FISH and could have progressed to a copy-number high phenotype. Most dedifferentiated carcinomas belonged to the hypermutated group, whereas pure undifferentiated carcinomas shared molecular genetic alterations with copy-number low or copy-number high tumors. The results indicate that undifferentiated and dedifferentiated endometrial carcinomas are molecularly heterogeneous tumors, which may have prognostic value.

Rosa-Rosa JM, Leskelä S, Cristóbal-Lana E, et al. Molecular genetic heterogeneity in undifferentiated endometrial carcinomas. *Mod Pathol.* 2016;29:1390–1398.

Correspondence: Dr. J. M. Rosa-Rosa at juan_manuel.rosa@salud.madrid.org or Dr. J. Palacios at jose.palacios@salud.madrid.org

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Correlation of prenatal diagnosis and findings after D&E for fetal anomalies

Despite increased use of dilation and evacuation for fetuses with developmental anomalies, the pathologic examination of fragmented specimens obtained by this technique has been understudied. The authors conducted a study to correlate pathologic findings in second trimester fetal dilation and evacuation (D&E) specimens with prenatal diagnoses established through ultrasound or chromosome studies, or both, to determine the value of pathologic examination for supplementing or correcting clinical diagnoses. In this retrospective study, clinical and pathologic findings were correlated in 448 D&E specimens performed for second trimester termination of pregnancy for fetal anomalies discovered on ultrasound examination (278 cases) or chromosome analysis (170 cases). In 109 of the 170 (64 percent) cases with chromosomal abnormalities, pathologists identified at least one congenital defect associated with the respective karyotype. In 278 cases with ultrasound-detected anomalies, pathologists confirmed the major congenital defect in 116 (42 percent) fetal specimens. Evaluating for congenital central nervous system and body wall/diaphragm pathologic findings proved challenging due to tissue disruption. However, taking all categories into account, pathologic studies corrected ultrasound diagnoses in 152 of 413 (37 percent) cases and yielded additional diagnostic findings in 137 (33 percent) cases. The authors concluded that in

a substantial number of cases, examination of fragmented fetuses corrected or refined prenatal diagnoses, demonstrating a role for detailed pathologic examination of D&E specimens in the quality control of prenatal imaging studies and, potentially, in aiding subsequent genetic counseling.

Boecking CA, Drey EA, Kerns JL, et al. Correlation of prenatal diagnosis and pathology findings following dilation and evacuation for fetal anomalies. *Arch Pathol Lab Med.* 2017;141:267–273.

Correspondence: Dr. Walter E. Finkbeiner at walter.finkbeiner@ucsf.edu