Anatomic Pathology Abstracts, 12/16

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.

Reproducibility of NEPTUNE descriptor-based scoring system with various types of images

Intravenous leiomyomatosis: expression profiling and molecular cytogenetic analyses

Morphological and molecular approach to synchronous NSCLC: impact on staging

Genetic alterations in triple-negative breast cancer identified by NGS

Classification of extraovarian implants in ovarian serous borderline tumor patients

Reproducibility of NEPTUNE descriptor-based scoring system with various types of images

The multicenter Nephrotic Syndrome Study Network, or NEPTUNE, digital pathology scoring system uses a novel and comprehensive methodology to document pathologic features from whole-slide images, immunofluorescence, and ultrastructural digital images. The authors conducted a study to estimate inter- and intra-reader concordance of this descriptor-based approach. They collected data from 12 pathologists (eight NEPTUNE and four non-NEPTUNE) with experience ranging from training to 30 years. The authors produced a descriptor reference manual and implemented a webinar-based protocol for consensus/cross-training. Intra-reader concordance for 51 glomerular descriptors was evaluated on jpeg images by seven NEPTUNE pathologists scoring 131 glomeruli three times—tests one, two, and three—with each test following a consensus webinar review. Inter-reader concordance of glomerular descriptors was evaluated in 315 glomeruli by all pathologists, while interstitial fibrosis and tubular atrophy (244 cases, whole-slide images) and four ultrastructural podocyte descriptors (178 cases, jpeg images) were evaluated once by six and five pathologists, respectively. Cohen's kappa for inter-reader concordance for 48 of 51 glomerular descriptors with sufficient observations was moderate (0.40<kappa≤0.60) for 17 and good (0.60<kappa≤0.80) for eight, for 52 percent with moderate or better kappas. Clustering of glomerular descriptors based on similar pathologic features improved concordance. Concordance was independent of years of experience and increased with webinar cross-training. Excellent concordance was achieved for interstitial fibrosis and tubular atrophy. Moderate-to-excellent concordance was achieved for all ultrastructural podocyte descriptors, with good-to-excellent concordance for descriptors commonly used in clinical practice, foot process effacement, and microvillous transformation. The NEPTUNE digital pathology scoring system enables novel morphologic profiling of renal structures. Moderate-to-excellent concordance was seen for 31 of 54 (57 percent) histologic and ultrastructural descriptors tested with sufficient observations. Descriptors not sufficiently represented require further testing. This study proffers the NEPTUNE digital pathology scoring system as a model for standardization of renal biopsy interpretation that can be extended outside the NEPTUNE consortium, enabling international collaborations.

Barisoni L, Troost JP, Nast C, et al. Reproducibility of the NEPTUNE descriptor-based scoring system on whole-slide images and histologic and ultrastructural digital images. *Mod Pathol.* 2016;29:671–684.

Correspondence: Dr. L. Barisoni at lbarisoni@med.miami.edu or Dr. B. W. Gillespie at bgillesp@umich.edu

[hr]

Intravenous leiomyomatosis: expression profiling and molecular cytogenetic analyses

Intravenous leiomyomatosis is an unusual smooth muscle neoplasm with quasi-malignant intravascular growth but a histologically banal appearance. The authors reported on the expression and molecular cytogenetic analyses of a series of 12 intravenous leiomyomatosis cases to better understand the pathogenesis of intravenous leiomyomatosis. All cases were analyzed for expression of HMGA2, MDM2, and CDK4 proteins by immunohistochemistry based on the authors' previous finding of der(14)t(12;14)(q14.3;q24) in intravenous leiomyomatosis. Seven of 12 (58 percent) intravenous leiomyomatosis cases expressed HMGA2, and none expressed MDM2 or CDK4. Colocalization of hybridization signals for probes from the HMGA2 locus (12q14.3) and from 14q24 by interphase FISH was detected in a mean of 89.2 percent of nuclei in HMGA2-positive cases by immunohistochemistry, but in only 12.4 percent of nuclei in negative cases. This indicated an association between HMGA2 expression and this chromosomal rearrangement ($P = 8.24 \times 10^{-10}$).
Four HMGA2-positive cases had more than two *HMGA2* hybridization signals per cell. No cases showed loss of a hybridization signal by interphase FISH for the frequently deleted region of 7q22 in uterine leiomyomata. One intravenous leiomyomatosis case analyzed by array comparative genomic hybridization revealed complex copy number variations. Finally, expression profiling was performed on three intravenous leiomyomatosis cases. Interestingly, hierarchical cluster analysis of the expression profiles revealed segregation of the intravenous leiomyomatosis cases with leiomyosarcoma rather than with myometrium, uterine leiomyoma of the usual histological type, or plexiform leiomyoma. These findings suggest that intravenous leiomyomatosis cases share some molecular cytogenetic characteristics with uterine leiomyoma and expression profiles similar to that of leiomyosarcoma cases, further supporting their intermediate, quasi-malignant behavior.

Ordulu Z, Nucci MR, Dal Cin P, et al. Intravenous leiomyomatosis: an unusual intermediate between benign and malignant uterine smooth muscle tumors. *Mod Pathol.* 2016;29:500–510.

Correspondence: Dr. B. J. Quade at bquade@bwh.harvard.edu

[hr]

Morphological and molecular approach to synchronous NSCLC: impact on staging

Distinguishing between multiple primary cancers and intrapulmonary metastases in patients with synchronous multifocal lung cancer can be challenging. It has been suggested that histological and genotypic assessment of multifocal lung tumors may influence staging. The authors conducted a study to determine the role of morphology and genotype in staging of surgically treated multifocal non-small cell lung carcinoma. Synchronous lung cancers from 60 patients (42 with adenocarcinoma and 18 with squamous cell carcinoma) that were clinically considered to represent intrapulmonary metastases were histologically subtyped according to the 2015 World Health Organization classification of lung tumors and subjected to genotypic analysis—*KRAS, EGFR, BRAF, PIK3CA, ALK, MET,* and *ROS1* in adenocarcinoma and *PIK3CA* and p16 in squamous cell carcinoma. Concordance between clinical criteria and histological subtyping was identified in about 50 percent of cases ($P<.0001$). Based on genotype, 44 percent of adenocarcinomas and 60 percent of squamous cell carcinomas with identified molecular alterations were considered to be intrapulmonary metastases. Concordance between histological and molecular staging was observed in 89 percent of adenocarcinomas and 56 percent of squamous cell carcinomas. Univariate survival analyses failed to demonstrate significant differences in overall or cancer-specific survival in patients with adenocarcinoma and squamous cell carcinomas restaged according to histology or molecular profile, or both. Lymph node metastases (N1/N2 versus N0; $P=.03$) and age greater than 65 years ($P=.05$) were associated with shorter overall survival rates. Furthermore, patients with squamous cell carcinomas with *p16* deletion showed shorter overall survival than those with squamous cell carcinomas without *p16* deletion ($P=.05$). No correlation was found between other molecular alterations, clinicopathological characteristics, and prognosis. The authors concluded that this study demonstrates that a comprehensive genotypic and morphologic assessment of surgically

treated multifocal lung cancers is feasible but not sufficient to establish their clonal relationship and prognosis.

Schneider F, Derrick V, Davison JM, et al. Morphological and molecular approach to synchronous non-small cell lung carcinomas: impact on staging. *Mod Pathol.* 2016;29:735–742.

Correspondence: Dr. S. Dacic at dacics@upmc.edu

[hr]

Genetic alterations in triple-negative breast cancer identified by NGS

Triple-negative breast cancer represents a heterogeneous group of breast carcinomas at both the histologic and genetic level. Although recent molecular studies have comprehensively characterized the genetic landscape of these tumors, few have a detailed histologic examination integrated into the analysis. The authors defined the genetic alterations in 39 triple-negative breast cancers using a high-depth targeted massively parallel sequencing assay and correlated the findings with a detailed morphologic analysis. They obtained representative frozen tissue of primary triple-negative breast cancers from patients treated at their institution between 2002 and 2010. They characterized tumors based on histologic subtype and morphologic features. DNA was extracted from paired frozen primary tumor and normal tissue samples and was subjected to a targeted massively parallel sequencing platform comprising 229 cancer-associated genes common across all experiments. The average number of nonsynonymous mutations was three (range, 0 – 10) per case. The most frequent somatic alterations were mutations in *TP53* (74 percent) and *PIK3CA* (10 percent) and *MYC* amplifications (26 percent). Triple-negative breast cancers with apocrine differentiation less frequently harbored TP53 mutations (25 percent) and *MYC* gains (none), and they displayed a high mutation frequency in *PIK3CA* and other *PI3K* signaling pathway-related genes (75 percent). Using a targeted massively parallel sequencing platform, the authors identified the key somatic genetic alterations previously reported in triple-negative breast cancers. Furthermore, their findings showed that triple-negative breast cancers with apocrine differentiation constitute a distinct subset characterized by a high frequency of PI3K pathway alterations similar to luminal subtypes of breast cancer.

Weisman PS, Ng CKY, Brogi E, et al. Genetic alterations of triple negative breast cancer by targeted next-generation sequencing and correlation with tumor morphology. *Mod Pathol.* 2016;29:476–488.

Correspondence: Dr. H. Y. Wen at weny@mskcc.org

[hr]

Classification of extraovarian implants in ovarian serous borderline tumor patients

The classification of extraovarian disease into invasive and noninvasive implants predicts patient outcome in patients with high-stage ovarian serous borderline tumors, which are tumors of low malignant potential. However, the morphologic criteria used to classify implants vary between studies. The authors conducted a study to compare the prognostic significance of various criteria. For the study, three pathologists independently evaluated peritoneal or lymph node implants, or both, from 181 patients with high-stage serous borderline tumors. The implants were evaluated for the morphologic features of micropapillary architecture, glandular architecture, nests of epithelial cells with surrounding retraction artifact set in densely fibrotic stroma, low-power destructive tissue invasion, single eosinophilic epithelial cells within desmoplastic stroma, mitotic activity, nuclear pleomorphism, and nucleoli. Follow-up of 156 (86 percent) patients ranged from 11 to 264 months (mean, 89 months; median, 94 months). Implants with low-power destructive invasion into underlying tissue were the best predictor of adverse patient outcome, with 69 percent overall survival rates and 59 percent disease-free survival rates ($P<.01$). In the evaluation of individual morphologic features, the low-power destructive tissue invasion criterion also had excellent reproducibility between observers ($\kappa = 0.84$). Extraovarian implants with micropapillary architecture or solid nests with clefts were often associated with tissue invasion but did not add significant prognostic value beyond

destructive tissue invasion alone. Implants without attached normal tissue were not associated with adverse outcome and appeared to be noninvasive. Because the presence of invasion in an extraovarian implant is associated with an overall survival rate analogous to that of low-grade serous carcinoma, the designation of low-grade serous carcinoma is recommended. Even though the low-power destructive tissue invasion criterion has excellent interobserver reproducibility, it is further recommended that the presence of an invasive implant be confirmed by at least two pathologists—preferably at least one of whom is an experienced gynecologic pathologist—to establish the diagnosis of low-grade serous carcinoma.

McKenney JK, Gilks CB, Kalloger S, et al. Classification of extraovarian implants in patients with ovarian serous borderline tumors (tumors of low malignant potential) based on clinical outcome. *Am J Surg Pathol.* 2016;40:1155–1164.

Correspondence information not provided.