Anatomic Pathology Selected Abstracts, 6/13

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Prolonged cold ischemia time and ER immunohistochemistry in breast cancer

To aid detection of estrogen receptor expression in breast tumors, the American Society of Clinical Oncology and College of American Pathologists recommend that cold ischemia time be kept under one hour. However, data to address the upper threshold of cold ischemia time are limited. Although it is routine practice at the authors' institution to keep cold ischemia time under one hour for breast core biopsy specimens, this is difficult for surgical specimens because of the comprehensive intraoperative assessment performed at the authors' institution. The authors conducted a retrospective study in which they compared estrogen receptor (ER) immunohistochemical staining results in paired breast tumor core biopsy specimens and resection specimens with cold ischemia times ranging from 64 to 357 minutes in 97 patients. The staining category (10 percent or more, positive; one percent to nine percent, low positive; less than one percent, negative) between the core biopsy and resection specimens changed for five patients (five percent). The weighted kappa statistic for ER staining category between the two specimen types was 0.86 (95 percent confidence interval, 0.74–0.99), indicating good concordance. The difference in the percentage of ER staining between core biopsy and resection was not significantly associated with cold ischemia time (P=.81, Spearman correlation). Although the authors did not observe significant associations between the difference in ER staining in the two specimen types and cold ischemia time after placing the patients in the three groups of "increase," "decrease," and "no change" using a difference of 25 percent in ER staining percentage as the cutoff, a trend of decreased ER staining with cold ischemia time of more than two hours was detected. No statistically significant association was found between change of ER staining and history of neoadjuvant chemotherapy. These findings indicate that a prolonged cold ischemia time of up to four hours (97 percent of this cohort) in the practice setting of the authors' institution has minimal clinical impact on ER immunohistochemical expression in breast tumors.

Li X, Deavers MT, Guo M, et al. The effect of prolonged cold ischemia time on estrogen receptor immunohistochemistry in breast cancer. *Mod Pathol.* 2013;26:71–78.

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Interobserver reliability in diagnosis of cartilaginous tumors in patients with multiple osteochondromas

Distinguishing between benign and malignant cartilaginous tumors located peripherally in the bone may be a challenging task in surgical pathology. The authors conducted a study to investigate interobserver reliability in the histological diagnosis of cartilaginous tumors in the setting of multiple osteochondromas and to evaluate possible histological parameters that could differentiate among osteochondroma and low- and high-grade secondary peripheral chondrosarcoma. For the study, 12 specialized bone-tumor pathologists assessed interobserver reliability in a set of 38 cases. Substantial agreement on diagnosis among all the reviewers was observed (intraclass correlation coefficient, 0.78). This study confirmed that mitotic figures and nuclear pleomorphism are hallmarks of high-grade secondary peripheral chondrosarcoma. However, despite substantial agreement, the authors demonstrated that histology alone cannot distinguish osteochondroma from low-grade secondary peripheral chondrosarcoma in the setting of multiple osteochondromas, as nodularity, presence of binucleated cells, irregular calcification, cystic/mucoid changes, and necrosis were not helpful in indicating malignant transformation of an osteochondroma. On the other hand, among the concordant cases, the cartilage cap in

osteochondroma was significantly less thick than in low- and high-grade secondary peripheral chondrosarcoma. Therefore, the authors concluded that their study shows that a multidisciplinary approach that integrates clinical and radiographical features and the size of the cartilaginous cap, in combination with a histological assessment, are crucial to the diagnosis of cartilaginous tumors.

De Andrea CE, Kroon HM, Wolterbeek R, et al. Interobserver reliability in the histopathological diagnosis of cartilaginous tumors in patients with multiple osteochondromas. *Mod Pathol.* 2012;25:1275–1283.

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High proliferation associated with inferior outcome in male breast cancer patients

Assessment of proliferation is important in female breast cancer, and treatment decisions are based on its results, especially in the luminal subgroups. Gene-expression analyses fail to group male breast cancer into the intrinsic subgroups established for female breast cancer. Even though proliferation has been shown to divide male breast cancer into molecular subgroups with different prognoses, the clinical importance of proliferation markers has not been elucidated. Previous studies of male breast cancer have demonstrated contradictory results regarding the prognostic impact of histological grade and Ki-67, parameters strongly associated with proliferation. The authors studied proliferation in male breast cancer by assessing other proliferation-related markers, namely cyclins A, B, and D1, and mitotic count. They investigated 197 male breast cancer cases with accessible paraffin-embedded material and outcome data and performed immunohistochemical stainings on tissue microarrays. Kaplan-Meier estimates and Cox proportional regression models were used for survival analyses, with breast cancer death as the event. The subset of patients with high expression of cyclin A (hazard ratio [HR], 3.7; P=.001) and B (HR, 2.7; P=.02) demonstrated a poorer survival. Furthermore, high mitotic count was associated with an increased risk of breast cancer death (HR, 2.5; P=.01). In contrast, cyclin D1 overexpression was predictive of better breast cancer survival (HR, 0.3; P=.001). The authors concluded that high levels of cyclin A and B expression and an elevated mitotic count result in a two- to threefold higher risk for breast cancer death, whereas cyclin D1 overexpression halves the risk. The clinical utility of these proliferation markers needs to be clarified further.

Nilsson C, Koliadi A, Johansson I, et al. High proliferation is associated with inferior outcome in male breast cancer patients. *Mod Pathol.* 2013;26:87–94.

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Relevance of number of examined lymph nodes in gastric cancer

The seventh edition of the tumor, lymph node, metastasis staging system increased the required number of examined lymph nodes in gastric cancer from 15 to 16. However, the same staging system defines lymph node-negative gastric cancer regardless of the number of examined nodes. The authors conducted a study in which they evaluated whether gastric cancer can be staged properly with fewer than 15 examined lymph nodes. They analyzed the survival rates of 10,010 patients who underwent curative gastrectomy from 1987 to 2007. The patients were divided into two groups according to the number of examined lymph nodes: an “insufficient” group (15 or fewer examined nodes) and a “sufficient” group (16 or more examined nodes). The survival curves of patients from both groups were compared according to the seventh edition of the tumor-node-metastasis (TNM) classification. Three hundred sixteen patients (3.2 percent) had 15 or fewer examined lymph nodes for staging after they underwent standard, curative lymphadenectomy. The authors found that patients who had T1 tumor classification, N0 lymph node status, and stage I disease with an insufficient number of examined lymph nodes after curative gastrectomy had a significantly worse prognosis than patients who had 16 or more examined nodes. Moreover, having an insufficient number of examined lymph nodes was an independent prognostic factor for patients who had T1, N0, and stage I disease. The authors concluded that lymph node-negative cancers in which 15 or fewer lymph nodes were examined, classified as N0 in the new TNM staging system, could not adequately predict patient survival after curative gastrectomy, especially in patients with early stage gastric cancer.

Son T, Hyung WJ, Lee JH, et al. Clinical implication of an insufficient number of examined lymph nodes after curative resection for gastric cancer. *Cancer.* 2012;118:4687–4693.

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Survival, morbidity, and cost associated with lymphadenectomy in low-risk endometrial cancer

Since 1999, patients with low-risk endometrial cancer, as defined by criteria set by the Mayo Clinic, have preferably not undergone lymphadenectomy (LND) at the Mayo Clinic. The authors prospectively assessed survival, sites of recurrence, morbidity, and cost in this low-risk cohort. Cause-specific survival was estimated using the Kaplan-Meier method and compared using the log-rank test. Complications were graded per the Accordion Severity Grading System. Thirty-day cost analyses were expressed in 2010 Medicare dollars. The authors found that among 1,393 consecutive surgically managed cases, 385 (27.6 percent) met inclusion criteria, accounting for 34.1 percent of type I endometrial cancer. There were 80 lymphadenectomy (LND) and 305 non-LND cases. Complications in the first 30 days were significantly more common in the LND cohort (37.5 percent versus 19.3 percent; P<.001). The prevalence of lymph node metastasis was 0.3 percent (one in 385). Over a median followup of 5.4 years, only five of 31 deaths were due to disease. The five-year cause-specific survival in LND and non-LND cases was 97.3 percent and 99 percent, respectively (P=.32). None of the 11 total recurrences occurred in the pelvic or para-aortic nodal areas. Median 30-day cost of care was $15,678 for LND cases and $11,028 for non-LND cases (P<.001). The estimated cost per upstaged low-risk case was $327,866 to $439,990, adding an additional $1,418,189 if all 305 non-LND cases had undergone LND. The authors concluded that lymphadenectomy dramatically increases morbidity and cost of care without discernible benefits in low-risk endometrial cancer, as defined by the Mayo criteria. In these low-risk patients, hysterectomy with salpingo-oophorectomy alone is appropriate surgical management and should be the standard of care.

Dowdy SC, Borah BJ, Bakkum-Gamez JN, et al. Prospective assessment of survival, morbidity, and cost associated with lymphadenectomy in low-risk endometrial cancer. *Gyn Oncol.* 2012;127:5–10.

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Role of Ki-67 proliferative index in predicting survival for patients with pulmonary carcinoid tumors

Pulmonary carcinoid tumors are classified as typical or atypical based on the mitotic index (two per 10 high-power fields) or the presence of necrosis, or both. Following incorporation of the Ki-67 index into the classification of gastrointestinal carcinoid tumors, the oncologists at the authors' institution have been requesting this test as part of the workup of pulmonary carcinoid tumors, although no established criteria exist for interpreting Ki-67 index in these neoplasms. The authors used the Ariol SL50 image analyzer to measure the Ki-67 index in 101 pulmonary carcinoid tumors (78 typical and 23 atypical) and then correlated the Ki-67 index and the histological diagnoses in univariate and multivariable analyses with overall survival. The mean Ki-67 indices for the typical carcinoids (3.7 standard deviation ± 4.0) and the atypical carcinoids (18.8 standard deviation ± 17.1) were significantly different (P<.001), although the frequency distributions of Ki-67 indices in the two groups overlapped considerably. Receiver operating characteristic curve analysis showed that a Ki-67 index cutoff value of five percent provided the best fit for specificity and sensitivity in predicting overall survival. Histological diagnosis and the Ki-67 index cutoff of five percent were each independently strong predictors of survival (P<.001 and P=.003, respectively). When considered together in multivariable analysis, histological diagnosis was the stronger predictor of overall survival, and a Ki-67 index cutoff of five percent did not provide additional significant predictive survival information within the typical or atypical carcinoid patient groups. A few typical carcinoid patients with Ki-67 indices of five percent appeared to have worse survival after five years than those with Ki-67 indices of less than five percent, but the data set was insufficiently powered to further analyze this finding. These findings do not provide best evidence for

routine use of the Ki-67 index to prognosticate overall short-term survival in patients with pulmonary carcinoid tumors.

Walts AE, Ines D, Marchevsky AM. Limited role of Ki-67 proliferative index in predicting overall short-term survival in patients with typical and atypical pulmonary carcinoid tumors. *Mod Pathol.* 2012;25:1258–1264.

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Grading neuroendocrine tumors using Ki-67 proliferation index

Gastrointestinal and pancreatic neuroendocrine tumors arise from disseminated neuroendocrine cells, expressing general and specific neuroendocrine markers. The World Health Organization 2010 classification of neuroendocrine tumors (NETs) is based on grading them according to the proliferation index, which is determined by immunohistochemical staining of the nuclear antigen Ki-67. The classification introduces Ki-67 as the most important criterion for tumor grading, influencing patients' prognoses and choice of treatment. The authors conducted a study to assess proliferation index value in NETs and its influence on tumor grading. The tumor material consisted of 51 NETs from the pancreas (n=31) and ileum (n=20). The slides were stained with the Ki-67 antibody and visualized using a polymer kit. Proliferation index was assessed visually by microscope oculars and using the public-domain, image-analysis software ImmunoRatio. The proliferation index was measured from the most proliferative areas of the tumor. The index values and tumor grade by ImmunoRatio were highly reproducible as compared with conventional assessment, which suffered from variation, especially if ascertained by different observers. Computer-aided assessments had almost perfect correlation (r=0.985, r=0.987, and r=0.995; P=.000) and reproducibility (k=0.886, k=0.886, and k=1.000; P=.000) in proliferation index values and tumor grades, respectively. The authors concluded that the proliferation index values and tumor grade between conventional and ImmunoRatio assessments by a qualified observer were in good agreement. ImmunoRatio is a qualified diagnostic aid to more objectively analyze Ki-67 proliferation index-based tumor grade in NETs.

Remes SM, Tuominen VJ, Helin H, et al. Grading of neuroendocrine tumors with Ki-67 requires high-quality assessment practices. *Am J Surg Pathol.* 2013;36(9):1359–1363.

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Dermal hypersensitivity reaction: a PCR-confirmed pattern of herpetic dermatitis

Herpetic dermatitis, whether due to herpes simplex virus (HSV) or varicella zoster virus, can present with similar clinical and histopathologic features. Further confounding matters, viral cytopathic changes are not always observed in biopsy specimens. Therefore, use of polymerase chain reaction (PCR) analysis can play an integral role in the definitive diagnosis of herpetic dermatitis and in distinguishing HSV I/HSV II from varicella zoster virus (VZV). The authors performed a study in which 40 patients with skin biopsies from 2004 to 2011 had PCR analysis performed to detect HSV I/II or VZV. Patient demographics, clinical impression, and histopathologic characteristics were reviewed and correlated with PCR findings. The authors found that, overall, complete correlation between clinical impression, histopathology, and PCR results was noted in 21 of 40 cases. In 19 cases, clinical impression and histopathology were discrepant, and in 15 of these cases PCR confirmed HSV or VZV infection. The authors also described three cases of herpetic dermatitis without viral change that histopathologically demonstrate the pattern of a dermal hypersensitivity reaction. The results of this study suggest that routine use of PCR for the definitive diagnosis of herpetic dermatitis should be considered when there is a clinical suspicion of herpes virus infection, even when specific histopathologic findings are lacking. Additionally, a dermal hypersensitivity reaction should be recognized as one histopathologic manifestation of herpes incognito.

Kinonen CL, Gleason BC, Thomas AB, et al. Dermal hypersensitivity reaction: a PCR-confirmed pattern of herpetic dermatitis. *J Cutan Pathol.* 2012;39(10):929–935.

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Ramifications of urologists' self-referral for pathology of biopsy specimens

Federal law allows physicians, in some circumstances, to refer patients for additional services to a facility in which the physician has a financial interest. The practice of physician self-referral for imaging and pathology services has been criticized because it can lead to increased use and escalating health care expenditures, with little or no benefit to patients. The author conducted a study in which she examined Medicare claims for men in a set of geographically dispersed counties to determine how the "in-office ancillary services" exception affected the use of surgical pathology services and cancer-detection rates associated with prostate biopsies. The author found that self-referring urologists billed Medicare for 4.3 more specimens per prostate biopsy than the adjusted mean of six specimens per biopsy that non-self-referring urologists sent to independent pathology providers, a difference of almost 72 percent. Additionally, the regression-adjusted cancer detection rate in 2007 was 12 percentage points higher for men treated by urologists who did not self-refer. This suggests that financial incentives prompt self-referring urologists to perform prostate biopsies on men who are unlikely to have prostate cancer. These results support closing the loophole that permits self-referral to in-office pathology laboratories.

Mitchell JM. Urologists' self-referral for pathology of biopsy specimens linked to increased use and lower prostate cancer detection. Health Affairs. 2012;31(4):741–749.

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