Anatomic Pathology Selected Abstracts, 7/13

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Intraoperative pathologic examination in the era of molecular testing for differentiated thyroid cancer

Diagnostic thyroidectomy is typically indicated for indeterminate thyroid cytology results. Traditionally, intraoperative pathologic examination (IOPE) helped guide the extent of initial surgery. Preoperative molecular testing of fine-needle aspiration cytology has emerged as another diagnostic adjunct, is highly specific for thyroid cancer, and can lead to appropriate initial total thyroidectomy. The authors hypothesized that preoperative molecular testing obviates the need for routine IOPE during lobectomy. In a retrospective, consecutive cohort study, they compared the outcomes of 670 patients undergoing thyroidectomy. Cohort A (January 2005 to December 2006) received surgery without molecular testing, and cohort B (January 2008 to September 2010) underwent preoperative molecular testing for BRAF, RAS, RET/PTC, and PAX8/PPARg mutations, as well as cytology assessment by the 2007 modified Bethesda criteria. In both cohorts, IOPE was performed during lobectomy, and a positive result prompted total thyroidectomy. The authors found that in cohort B, total thyroidectomy was more often the initial surgery (B 62 percent versus A 45 percent; P=.001), and a positive molecular test result was the only factor prompting initial total thyroidectomy in 18 (nine percent) patients. Among 315 patients who had initial lobectomy, thyroid cancer was infrequently diagnosed by IOPE in both cohorts (A 3.6 percent versus B 1.7 percent; P=.5). The sensitivity of IOPE in detecting differentiated thyroid cancer of 1 cm or greater decreased by more than 60 percent with routine use of molecular testing and the Bethesda criteria (A 18.4 percent versus B 5.9 percent). After lobectomy, differentiated thyroid cancer of 1 cm or greater was equally likely to be diagnosed in both cohorts (P=.1), but follicular variant papillary thyroid cancer was more common in cohort B (B 74 percent versus A 45 percent; P=.02). The authors concluded that together with the Bethesda cytologic criteria, preoperative molecular testing allows for an increased rate of initial definitive total thyroidectomy and eliminates the need for routine intraoperative pathologic examination during diagnostic lobectomy.

McCoy KL, Carty SE, Armstrong MJ, et al. Intraoperative pathologic examination in the era of molecular testing for differentiated thyroid cancer. *J Am Coll Surg*. 2012;215:546–554.
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Clinical outcome of atypical endometrial hyperplasia diagnosed on endometrial biopsy

The authors conducted a study to review the rate of concurrent endometrial cancer in patients with a preoperative diagnosis of atypical endometrial hyperplasia (AEH) and to determine the features of concurrent endometrial carcinoma and their impact on the subsequent management of AEH. They reviewed a retrospective series of 219 AEHs diagnosed locally in routine practice over 24 years and followed by a repeat biopsy or hysterectomy. A series of 65 cases with a malignant diagnosis on preoperative sampling served as a control group. The authors obtained clinicopathologic parameters and collected and analyzed published data on the risk of malignancy and features of malignant tumors after a diagnosis of AEH. The study also reported on 2,571 patients diagnosed in 31 additional published studies. This showed a wide variation in the positive predictive value (PPV) of AEH for detecting endometrial cancer (six percent to 63 percent), with an overall PPV of 37 percent. This variation is not only based on the differences among studies, but also on the degree of atypia (mild/moderate [PPV, 13 percent] or severe [PPV, 50 percent]), type of subsequent intervention (biopsy versus hysterectomy), and, more importantly, time period of diagnosis (approximately 20 percent in studies published before the 1990s and up to 40 percent to 48 percent in recently published cases). Of the cases with a benign outcome, approximately 40 percent to 50 percent

showed AEH, with a potential risk of progressing to invasive carcinoma in 25 percent of cases. Malignant tumors after AEH diagnosis are associated with features of good prognosis with endometrioid morphology, lower grade, and early stage. Although the overall PPV of AEH is 37 percent, a figure of 40 percent to 48 percent is expected in cases currently diagnosed in routine practice. The authors concluded that providing qualifying criteria for AEH will help identify its various associated risks and therefore should be included in routine pathology reports whenever possible. Unless there is a clinical contraindication, hysterectomy should be performed to treat concurrent carcinoma and to reduce the risk of subsequent carcinoma in nonmalignant cases with residual AEH.

Rakha E, Wong SC, Soomro I, et al. Clinical outcome of atypical endometrial hyperplasia diagnosed on an endometrial biopsy: institutional experience and review of literature. *Am J Surg Pathol*. 2012;36(11):1683–1690.
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Evaluation of pleomorphic lobular carcinoma of the breast relative to histological grade

Pleomorphic lobular carcinoma is considered a biologically aggressive variant of invasive lobular carcinoma of the breast. However, there is no consensus on the definition and whether this subtype adds useful information to histological grade. The authors studied 202 grade two or three invasive lobular carcinomas. They categorized the tumors according to the components of histological grade: tubules, pleomorphism, and mitoses. Pleomorphic lobular carcinoma was defined as a carcinoma with a lobular growth pattern and marked nuclear pleomorphism (pleomorphism three). Breast cancer-specific survival was used to analyze prognosis. Grade three pleomorphic lobular carcinomas (tubules three, pleomorphism three, mitoses two, and tubules three, pleomorphism three, mitoses three) had a worse prognosis than grade two carcinomas (tubules three, pleomorphism two, mitoses one). Grade two lobular carcinomas with marked nuclear pleomorphism (tubules three, pleomorphism three, mitoses one) had a similar prognosis to grade two carcinomas with moderate pleomorphism (tubules three, pleomorphism two, mitoses one). Survival was associated with mitotic score but not with nuclear pleomorphism on univariate and multivariate analyses. A non-classical growth pattern was seen more frequently in all subgroups with marked nuclear pleomorphism and was associated with worse survival. Histological grade and nodal status were independent of prognostic factors. The authors concluded that histological grade, in particular the mitotic component, in invasive lobular carcinomas is of prognostic importance, but pleomorphic type does not provide additional useful prognostic information.

Rakha EA, van Deurzen CHM, Paish EC, et al. Pleomorphic lobular carcinoma of the breast: Is it a prognostically significant pathological subtype independent of histological grade? *Mod Pathol*. 2013;26:496–501.
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Diagnostic value of fungal fluorescence in onychomycosis

Fluorescence of pathogenic fungi has been previously shown when hematoxylin-and-eosin–stained sections were examined under a fluorescence microscope. The authors hypothesized that this phenomenon could aid in evaluating nail specimens for onychomycosis. They conducted a study in which 48 routinely stained nail sections of periodic acid-Schiff (PAS)–positive onychomycosis, along with 23 PAS-negative control specimens with a clinical diagnosis of onychomycosis, were analyzed under a fluorescence microscope to determine the clinical usefulness of this technique. In most of the cases, fluorescence of fungal organisms was noted. Fungi were identified by their tubular or annular shapes with fluorescence surrounding them. The sensitivity and specificity of the method were 96 percent and 90 percent, respectively. In some cases, it was difficult to identify the fungi because of the relative paucity of organisms, weak fluorescence, and high background fluorescence of eosinophilic nail keratin. The authors concluded that fluorescence microscopy can be used as a rapid screening tool for identifying fungi in nail specimens.

Idriss MH, Khalil A, Elston D. The diagnostic value of fungal fluorescence in onychomycosis. *J Cutan Pathol*. 2013;40(4):385–390.

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Distribution of microscopic melanoma metastases in sentinel lymph nodes: implications for pathology protocols

The authors investigated the utility of sectioning at multiple levels in the histopathologic analysis of sentinel lymph nodes for melanoma and the correlation of metastasis size with risk of subsequent metastasis. Metastatic melanoma was identified in sentinel lymph nodes (SLNs) from 91 of 475 (19 percent) melanoma patients who underwent SLN sampling at Massachusetts General Hospital between 2004 and 2008. All SLNs were evaluated by a nine-slide protocol: sets of MART-1, hematoxylin and eosin, and S100 stains at three distinct levels separated by 80 µm. The location and size of the tumor deposits were evaluated in the context of subsequent metastasis and overall survival. Of the 91 patients with positive sentinel nodes, all nine protocol slides were available for review in 61 (67 percent). Eleven of 61 patients had no tumor present in the first set of levels; two of these patients died of metastatic melanoma. Patients in whom 11 or more tumor cells were detected in the sentinel node had a greater chance of developing subsequent metastases when compared with patients in whom 10 or fewer tumor cells were detected (P=.05). Of those with either metastases greater than 2 mm in diameter or extracapsular extension, 50 percent developed metastases beyond the SLN basin. Eliminating one of the three levels in the SLN detection protocol would have led to a false-negative diagnosis in 18 percent of patients.

Lobo AZ, Tanabe KK, Luo S, et al. The distribution of microscopic melanoma metastases in sentinel lymph nodes: implications for pathology protocols. Am J Surg Pathol. 2012;36(12):1841–1848.
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Global mutational profiling of formalin-fixed colon cancers from a pathology archive

The advent of next-generation sequencing technologies, which significantly increase the throughput and reduce the cost of large-scale sequencing efforts, provides an unprecedented opportunity for discovery of novel gene mutations in human cancers. However, it remains a challenge to apply next-generation technologies to DNA extracted from formalin-fixed, paraffin-embedded cancer specimens. The authors described the development of a custom DNA capture method using next-generation for detecting 140 driver genes in five formalin-fixed, paraffin-embedded colon cancer samples using an improved extraction process to produce high-quality DNA. Isolated DNA was enriched for targeted exons and sequenced using the Illumina next-generation platform. An analytical pipeline using three software platforms to define single-nucleotide variants was used to evaluate the data output. Approximately 250 × average coverage was obtained, with more than 96 percent of target bases having at least 30 sequence reads. Results were then compared with previously performed high-throughput Sanger sequencing. Using an algorithm of needing a positive call from all three callers to give a positive result, 98 percent of the verified Sanger sequencing somatic driver gene mutations were identified by the authors' method with a specificity of 90 percent. In all, 13 insertions and deletions identified by next-generation sequencing were confirmed by Sanger sequencing. The authors also applied this technology to two components of a biphasic colon cancer, which had strikingly different histology. Remarkably, no new driver gene mutation accumulation was identified in the more undifferentiated component. Applying this method to profiling of formalin-fixed, paraffin-embedded colon cancer tissue samples yields sensitivity and specificity for mutation detection equivalent to Sanger sequencing of matched cell lines derived from these cancers. This method enables high-throughput comprehensive mutational profiling of colon cancer samples and can easily be extended to allow targeted sequencing from formalin-fixed, paraffin-embedded material from other tumor types.

Adams MD, Veigl ML, Wang Z, et al. Global mutational profiling of formalin-fixed human colon cancers from a pathology archive. *Mod Pathol*. 2012;25(12):1599–1608.

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