Anatomic pathology selected abstracts

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Study of hepatic pathology in patients dying of COVID-19 complications

February 2021—SARS-CoV-2 primarily causes pulmonary injury, but it has been implicated in hepatic injury through the use of serum markers and histologic evaluation. The histologic pattern of injury has not been completely described, and studies quantifying viral load in the liver are lacking. The authors conducted a study in which they reported the clinical and histologic findings related to the liver in 40 patients who died of complications of COVID-19. For the study, they subjected a subset of liver tissue blocks to polymerase chain reaction (PCR) for viral RNA. Peak levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated, with a median ALT peak of 68 U/L (normal up to 46 U/L) and median AST peak of 102 U/L (normal up to 37 U/L). Macrovesicular steatosis was the most common finding, involving 30 (75 percent) patients. Mild lobular necroinflammation and portal inflammation were present in 20 (50 percent) cases each. Vascular pathology, including sinusoidal microthrombi, was seen in only six (15 percent) cases. PCR of liver tissue was positive in 11 of 20 (55 percent) patients tested. The authors concluded that patients dying of COVID-19 had biochemical evidence of hepatitis of variable severity and demonstrated histologic findings of macrovesicular steatosis, mild acute hepatitis (lobular necroinflammation), and mild portal inflammation. Viral RNA was present in a sizeable subset of liver tissue samples.

Lagana SM, Kudose S, Iuga AC, et al. Hepatic pathology in patients dying of COVID-19: a series of 40 cases including clinical, histologic, and virologic data. *Mod Pathol*. 2020;33(11):2147–2155.

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DNA MMR and *MLH1* promoter methylation in endometrioid endometrial carcinoma

The pathogenesis of DNA mismatch repair-deficient endometrial carcinoma is driven by inactivating methylation or, less frequently, mutation of the key mismatch repair (MMR) genes *MLH1, PMS2, MSH2,* or *MSH6*. The authors conducted a study to evaluate the prognostic and clinicopathologic differences between methylation-linked and nonmethylated DNA MMR-deficient endometrioid endometrial carcinoma (EC). They performed MMR IHC and methylation-specific multiplex ligation-dependent probe amplification and classified 682 unselected endometrioid ECs as MMR proficient (MMRp, n = 438) and MMR deficient (MMRd, n = 244), with the latter subcategorized as methylated (MMRd Met) and nonmethylated tumors. Loss of MMR protein expression was detected in 35.8 percent of the tumors, with MLH1 + PMS2 in 29.8 percent, PMS2 in 0.9 percent, MSH2 + MSH6 in 1.3 percent, MSH6 in 2.8 percent, and multiple abnormalities in 0.9 percent. Of the 244 MMRd cases, 76 percent were methylation linked. MMR deficiency was associated with older age, high grade of differentiation (G3), advanced stage (II–IV), larger tumor size, abundant tumor-infiltrating lymphocytes, PD-L1 positivity in immune cells and combined positive score, wild-type p53, negative L1CAM, ARID1A loss, and type of adjuvant therapy. MMRd-Met phenotype correlated with older age and larger tumor size and predicted diminished disease-specific survival in the whole cohort. In the MMRd subgroup, univariate analysis demonstrated an association between disease-specific survival and disease stage II–IV, high grade (G3), deep myometrial invasion, lymphovascular invasion, estrogen receptor negativity, and L1CAM positivity. The authors concluded that MMR methylation profile correlates with clinicopathologic characteristics of endometrioid EC and MMRd-Met phenotype predicts lower disease-specific survival. MMR deficiency, but not *MLH1* methylation status, correlates with T-cell inflammation and PD-L1 expression.

Pasanen A, Loukovaara M, Bützow R. Clinicopathological significance of deficient DNA mismatch repair and *MLH1* promoter methylation in endometrioid endometrial carcinoma. *Mod Pathol*. 2020;33:1443–1452.

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Automated quantitation of CD8-positive T cells in colonic adenocarcinoma

Despite their association with DNA mismatch repair protein deficiency, colonic adenocarcinomas with mucinous, signet ring cell, or medullary differentiation have not been associated with improved survival when compared with conventional adenocarcinomas in most studies. Recent studies indicate that increased T-cell infiltration in the tumor microenvironment has a favorable prognostic effect in colonic adenocarcinoma. However, to the authors' knowledge, the prognostic effect of tumor-associated T cells has not been evaluated in histologic subtypes of colonic adenocarcinoma. Therefore, they evaluated CD8-positive T-cell density in 259 patients with colonic adenocarcinoma, including 113 patients with tumors demonstrating mucinous, signet ring cell, or medullary differentiation, using a validated automated quantitative digital image-analysis platform. The authors correlated CD8-positive T-cell density with histopathologic variables, DNA mismatch repair (MMR) status, molecular alterations, and survival. CD8-positive T-cell densities were found to be significantly higher for MMR protein-deficient tumors ($P<.001$), BRAF V600E mutant tumors ($P=.004$), and tumors with medullary differentiation ($P<.001$) but did not correlate with mucinous or signet ring cell histology ($P>.05$ for both). In the multivariable model of factors predicting disease-free survival, increased CD8-positive T-cell density was associated with improved survival in the entire cohort (hazard ratio, 0.34; 95 percent confidence interval [CI], 0.15–0.75; $P=.008$) and in an analysis of patients with tumors with mucinous, signet ring cell, or medullary differentiation (hazard ratio, 0.06; 95 percent CI, 0.01–0.54; $P=.01$). The prognostic effect of CD8-positive T-cell density was independent of tumor stage, MMR status, *KRAS* mutation, and *BRAF* mutation. Venous invasion was the only other variable independently associated with survival in the entire cohort and in patients with tumors with mucinous, signet ring cell, or medullary differentiation. In summary, the results indicate that the prognostic value of MMR protein deficiency is most likely attributed to increased tumor-associated CD8-positive T cells. Furthermore, automated quantitative CD8 T-cell analysis is a better biomarker of patient survival, particularly in patients with tumors demonstrating mucinous, signet ring cell, or medullary differentiation.

Hartman DJ, Frank M, Seigh L, et al. Automated quantitation of CD8-positive T cells predicts prognosis in colonic adenocarcinoma with mucinous, signet ring cell, or medullary differentiation independent of mismatch repair protein status. *Am J Surg Pathol*. 2020;44:991–1001.

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A systematic review of pathological findings in COVID-19

Three stages of COVID-19 have been identified—based on viral infection, pulmonary involvement with inflammation, and fibrosis. Moreover, low- and high-elastance phenotypes can be distinguished in mechanically ventilated patients based on lung mechanics, ventilation-to-perfusion ratio, and computed tomography scans. These phenotypes have presumed differences in their underlying pathophysiology. Although essential for therapeutic guidance, the pathophysiology of COVID-19 is poorly understood. Therefore, the authors systematically reviewed published case reports and case series in order to increase understanding of the pathophysiology of the disease by constructing a timeline and correlating histopathological findings with clinical stages of COVID-19. They used PRISMA-IPD (Preferred Reporting Items for a Systematic Review and Meta-analysis of Individual Participant Data) guidelines and included 42 articles reporting 198 individual cases in the analysis. The authors identified three main histological patterns in lung samples (n=131): epithelial (n=110, 85 percent), with reactive epithelial changes and diffuse alveolar damage; vascular (n=76, 59 percent) with microvascular damage, (micro)thrombi, and acute fibrinous and organizing pneumonia; and fibrotic (n=28, 22 percent) with interstitial fibrosis. The epithelial and vascular patterns can present in all stages of symptomatic COVID-19, whereas the fibrotic pattern presents at approximately three weeks. Moreover, patients can present with more than one pattern simultaneously or consecutively. These findings are consistent with prior knowledge about clinical patterns of viral infection,

development of hyperinflammation and hypercoagulability, and fibrosis. Close collaboration among medical staff is necessary to translate this knowledge and classification of pathophysiological mechanisms into clinical stages of disease in patients. Moreover, additional research, including histopathological studies, is warranted to develop reliable, clinically relevant biomarkers by correlating pathological and other findings, thereby increasing understanding of COVID-19 and facilitating the transition to precision medicine.

Polak SB, Van Gool IC, Cohen D, et al. A systematic review of pathological findings in COVID-19: a pathophysiological timeline and possible mechanisms of disease progression. *Mod Pathol*. 2020;33(11):2128–2138.

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Use of microscopic size measurements in post-neoadjuvant therapy resections of pancreatic ductal adenocarcinomas

Pancreatic ductal adenocarcinomas are increasingly being treated with neoadjuvant therapy. However, American Joint Committee on Cancer (AJCC) eighth edition T staging based on tumor size does not reflect treatment effect, which often results in multiple small foci of residual tumor in a background of mass-forming fibrosis. The authors evaluated the performance of AJCC eighth edition T staging in predicting patient outcomes using a microscopic tumor size measurement method. They reviewed 106 post-neoadjuvant therapy pancreatectomies and measured all individual tumor foci. T stages based on gross size with microscopic adjustment (GS) and the largest single microscopic focus size (MFS) were examined in association with clinicopathological variables and patient outcomes. Sixty-three of 106 (59 percent) tumors were locally advanced; 78 percent received Folfirinox treatment. The average GS and MFS were 25 mm and 11 mm, respectively. Based on GS and MFS, respectively, nine cases each were classified as T0, 35 and 85 cases as T1, 42 and 12 cases as T2, and 20 and zero cases as T3. Higher GS- and MFS-based T stages were significantly associated with higher tumor regression grade, lymphovascular and perineural invasion, and higher N stage. Furthermore, higher MFS-based T stage was significantly associated with shorter disease-free survival ($P<.001$) and shorter overall survival ($P=.002$). GS was significantly associated with overall survival ($P=.046$) but not with disease-free survival. The authors concluded that in post-neoadjuvant therapy PDAC resections, MFS-based T staging is superior to GS-based T staging for predicting patient outcomes, suggesting that microscopic measurements have clinical utility beyond the conventional use of GS measurements alone.

Zhang ML, Kem M, Rodrigues C, et al. Microscopic size measurements in post-neoadjuvant therapy resections of pancreatic ductal adenocarcinoma (PDAC) predict patient outcomes. *Histopathol*. 2020;77(1):144–155.

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Appendageal tumors and tumor-like lesions of the testis and paratestis

The testicular hilum and paratestis contain several embryologically diverse anatomic structures, including the spermatic cord, tunica vaginalis, epididymis, rete testis, and other embryonic remnants. A number of benign and malignant lesions arise from these morphologically distinct structures, and, owing to their proximity, it is challenging to classify and subsequently stage these tumors. The authors conducted a retrospective review of the paratesticular appendageal and rete testis tumors and tumor-like lesions diagnosed in their pathology department from 1985 through 2016. They excluded soft-tissue lesions/tumors and identified 146 paratesticular appendageal and rete testis tumors and tumor-like lesions. Most tumors were benign (n=107; 73 percent). Adenomatoid tumor (26 percent) was the most common benign tumor, followed by different types of cysts (19 percent), mesothelial hyperplasia (18 percent), serous cystadenoma (5.5 percent), and rete testis adenoma (four percent). Malignant lesions composed 23 percent of the cases, with mesothelioma being the most common (15 percent), followed by adenocarcinoma of the rete testis (four percent), serous cystadenocarcinoma (two percent), and papillary and clear cell adenocarcinoma of the epididymis (two percent). Serous borderline tumors and melanotic neuroectodermal tumor (retinal anlage tumors) composed the remaining four percent of cases. The authors concluded that a wide range of benign and malignant lesions can arise from the paratesticular region. Awareness of these lesions and

their histologic spectrum is crucial to avoid diagnostic pitfalls and allows pathologists to establish a correct diagnosis and subsequent treatment plan.

Al-Obaidy KI, Alruwaii FI, Ulbright TM, et al. Appendageal tumors and tumor-like lesions of the testis and paratestis: a 32-year experience at a single institution. *Hum Pathol*. 2020;103:25–33. https://doi.org/10.1016/j.humpath.2020.06.006

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