GIPS Journal Watch May & June 2017

The Use of Ancillary Stains in the Diagnosis of Barrett Esophagus and Barrett Esophagus-associated Dysplasia: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society

Srivastava A, Appelman H, Goldsmith JD, Davison JM, Hart J, Krasinskas AM.
Am J Surg Pathol. 2017 May;41(5):e8-e21.
https://www.ncbi.nlm.nih.gov/pubmed/28248814

Eminent pathologists from the Rodger C. Haggitt Gastrointestinal Pathology Society Executive Committee provided recommendations for the use of the ancillary stains in the diagnosis of Barrett esophagus (BE) and BE associated dysplasia, based on a comprehensive and critical literature review. BE, a glandular metaplasia extending ≥1 cm proximal to the gastroesophageal junction (GEJ), is an important diagnosis because it is a risk factor for adenocarcinoma of the distal esophagus or GEJ. Controversies exist regarding the use of ancillary stains in the diagnosis of BE and BE associated dysplasia. The first question addressed: 'Are ancillary stains needed to diagnose BE?' And Insufficient evidence was found to justify reflexive use of Alcian blue (at pH 2.5) and/or periodic-acid Schiff stains on all esophageal biopsies to diagnose BE because goblet cells, if present, are almost always identifiable on routine H&E stained sections. However, these stains may be useful in distinguishing pseudogoblet cells from true goblet cells. The other conclusion was that mucin glycoprotein immunostains and markers of intestinal phenotype (CDX2, Das-1, villin, Hep Par 1, and SOX9) are not recommended to aid in the diagnosis of BE. The seconds questions addressed: 'Are ancillary stains needed for dysplasia diagnosis or risk stratification in patients with BE?' Many studies have shown that p53 immunostain can be useful in diagnosing dysplasia but can't be used separate low grade dysplasia (LGD) from high grade dysplasia (HGD). Abnormal p53 immunostaining is a risk factor for progression to HGD or carcinoma. However, its utility is limited by the lack of a widely accepted definition of p53 immunostaining and the absence of guidelines regarding how often and when p53 immunostaining should be performed. Expression of cyclin D1, AMACR, and IMP3, an oncofetal protein, and loss of SOX2 expression have all been reported in BE-associated HGD and esophageal adenocarcinomas but are not entirely specific for dysplasia and have variable sensitivity. MUC stains, MUC5AC and MUC2, CD10, and CDX2 may help in diagnosing foveolar-type dysplasia, but are not useful in the diagnosis of BE with and without dysplasia. Their conclusion was that the diagnosis of dysplasia in BE remains a morphologic diagnosis and that ancillary stains are not recommended at this time for risk BE stratification. This manuscript should be used to aid pathologists and gastroenterologists in diagnosing and managing patients with BE.

Predictors of Progression in Barrett's Esophagus with Low-Grade Dysplasia: Results from a Multicenter Prospective BE Registry.
Krishnamoorthi R, Lewis JT, Krishna M, Crews NJ, Johnson ML, Dierkhising RA, Ginos BF, Wang KK, Wolfsen HC, Fleischer DE, Ramirez FC, Buttar NS, Katzka DA, Iyer PG.
Am J Gastroenterol. 2017 Jun;112(6):867-873.
https://www.ncbi.nlm.nih.gov/pubmed/28374813

The authors of this study aimed to estimate the risk of progression in a well characterized cohort of Barrett's esophagus (BE) patients diagnosed with low-grade dysplasia (LGD), assess risk factors of progression to high-grade dysplasia (HGD) or adenocarcinoma (EAC), and estimate the effect of independent confirmation of LGD diagnosis by a panel of two expert GI pathologists on progression risk. A large registry of BE patients was examined for participants with inclusion criteria including individuals who had a histologic diagnosis of BE-LGD with more than 12 months follow-up. Individuals with a prior history of HGD/EAC were excluded as were patients who developed HGD/EAC within one year of the index LGD diagnosis, in keeping with the strict definition of progression. This resulted in a final cohort of 244 patients. Of these, 56 (22.9%) were diagnosed with HGD/EAC within a year of the index biopsy while 14 (5.7%) progressed to HGD/EAC after 12 months. Given this latter finding, the overall annual risk of progression was 1.2%. Re-evaluation of the available index slides by a panel of two expert GI pathologists resulted in 29.1% of cases being downgraded to negative for dysplasia while the remaining cases were considered LGD or indefinite for dysplasia upon review. These categories were considered equivalent by the authors for portions of their analysis. The risk of progression in the confirmed LGD group increased to 2.4% which the authors note is almost an eight times higher risk of progression over the original LGD group. Univariate analysis of a number of other clinical and endoscopic factors including age, sex, smoking history, BE length, and multifocality of LGD did not demonstrate any other factors contributing to the rates of progression in this cohort. The authors conclude that LGD is likely a marker for prevalent HGD/EAC making evaluation of these patients critical. The authors also suggest that independent review of LGD diagnosis by a panel of GI pathologists may be warranted in some situations.

Eosinophilic Esophagitis and Esophageal Granular Cell Tumor: An Unexpected Association.
Riffle ME, Polydorides AD, Niakan J, Chehade M.
Am J Surg Pathol. 2017 May;41(5):616-621.
https://www.ncbi.nlm.nih.gov/pubmed/28296675

This study determined the prevalence of esophageal eosinophilia and eosinophilic esophagitis (EoE) in patients with esophageal granular cell tumors (GCT). GCTs may be a reactive process, arising in areas of trauma or chronic inflammation and may represent a traumatic neuroma variant. 18 of 23 cases of esophageal GCTs diagnosed over 20 years were available for histologic review (16 adults, 2 pediatric). Both pediatric cases had confirmed EoE. Four of the 16 adult cases (25%) had concurrent esophageal eosinophilia, consistent with EoE. All esophageal GCTs showed diffusely positive cytoplasmic and nuclear staining for S100 protein. 12 of the 18 patients (67%) had eosinophils infiltrating the tumor itself (peak eosinophils, 1 to 16/HPF); and

all 6 patients with EoE also had eosinophils in the GCT. Two adult GCTs displayed atypical cytologic features, associated with malignant GCTs, reported in 2% to 4% of GCT lesions. Based on finding an unexpected association between EoE and esophageal GCTs both in children and adults, the authors proposed evaluating surrounding tissue for eosinophilia when esophageal GCT is diagnosed and adding GCT as a potential complication of untreated EoE.

Lanthanum deposition from oral lanthanum carbonate in the upper gastrointestinal tract.
Hoda RS, Sanyal S, Abraham JL, Everett JM, Hundemer GL, Yee E, Lauwers GY, Tolkoff-Rubin N, Misdraji J.
Histopathology. 2017 Mar;70(7):1072-1078.
https://www.ncbi.nlm.nih.gov/pubmed/28134986

Lanthanum carbonate is used to manage hyperphosphatemia in patients with renal failure. It results in lanthanum deposits in the gastroduodenal mucosa; 5 such cases are described in this report, 3 of which were confirmed on electron microscopy and energy dispersive X-ray analysis. One patient had deposits but had last taken lanthanum 7 years prior to biopsy. The deposition mimics other forms of drug injury in the GI tract, such as iron pill gastropathy. Clinical symptoms are varied, including dysphagia, epigastric burning, and early satiety among others. Endoscopically, the gastric antrum can appear erythematous or may show superficial ulcers. Microscopically, the deposits consist of lamina propria histiocytes with amphophilic granular material and coarser brown to deep purple material. The material is refractile but not birefringent on polarization. In 1 case, they formed vague aggregates resembling poorly formed granulomas. Antral biopsies shows atrophy or reactive gastropathy; fundic biopsies showed less dramatic changes, with 1 case showing foveolar hyperplasia. The duodenal biopsy studied shows lamina propria expansion by histiocytes, congestion of the villous tips, and focal foveolar metaplasia. Iron stain shows faint staining of the largest particles. PAS-D shows fait staining of the granular material. The long-term consequences of lanthanum deposits are unknown, but this is a useful diagnosis to be aware of in the appropriate clinical setting.

Massive gastric juvenile-type polyposis: a clinicopathological analysis of 22 cases.
Gonzalez RS, Adsay V, Graham RP, Shroff SG, Feely MM, Drage MG, Lewin DN, Swanson EA, Yantiss RK, Bagci P, Krasinskas AM.
Histopathology. 2017 May;70(6):918-928.
https://www.ncbi.nlm.nih.gov/pubmed/27991685

Massive gastric polyposis is a rare disease that is associated with juvenile polyposis syndrome (JPS). This study characterized lesions in 12 men and 10 women, 14 of whom carried a diagnosis of JPS and tended to present at a younger age (40 years versus 60 years without a known diagnosis of JPS). 18 patients had carpeting of the gastric mucosa by polyps ranging from a few mm to about 10 cm in size. Characteristic microscopic features include a smooth outer contour, prominent stromal edema, and widely spaced and cystically dilated glands lined

by foveolar epithelium. Background mucosa is normal. 4 cases (18%) contained adenocarcinoma and 7 (32%) had dysplasia. Patchy loss of SMAD4 is common, with 5 of 6 tested patients showing germline SMAD4 mutation. Pathologists should be aware of this polyposis syndrome.

Clinical significance of spasmolytic polypeptide-expressing metaplasia and intestinal metaplasia in Epstein-Barr virus–associated and Epstein-Barr virus–negative gastric cancer
Zhang Y, Chen JN, Dong M, Zhang ZG, Zhang YW, Wu JY, Du H, Li HG, Huang Y, Shao CK.
Hum Pathol. 2017 May;63:128-138.
https://www.ncbi.nlm.nih.gov/pubmed/28300576

Clinicopathologic features of 64 EBV+ and 154 EBV- treatment naïve gastric cancers were studied, in particular the frequency of gastric SPEM (Spasmolytic polypeptide-expressing metaplasia; more commonly referred to as pseudopyloric metaplasia, or mucous metaplasia, or antralization of the corpus) and intestinal metaplasia (IM), and the composition of any inflammatory infiltrate. The authors found that both EBV+ and EBV- tumors had a higher concurrence with SPEM as compared to IM. Advanced SPEM (defined as clusters of pyloric-like glands), however, correlated strongly with the presence of IM, and both were found more frequently in EBV- tumors. The inflammatory component in all gastric cancers were similar, with acute inflammation present in about 40-50% of cases, and chronic inflammation present in all. Patients with EBV+ gastric cancers had longer overall survival, but this was not influenced by differences in SPEM or IM. The authors suggest that because advanced SPEM is strongly associated with IM in EBV- cancers, SPEM should be considered a pre-cancerous metaplasia.

High Incidence of Celiac Disease in a Long-term Study of Adolescents With Susceptibility Genotypes
Liu E, Dong F, Barón AE, Taki I, Norris JM, Frohnert BI, Hoffenberg EJ, Rewers M.
Gastroenterology. 2017 May;152(6):1329-1336.e1.
https://www.ncbi.nlm.nih.gov/pubmed/28188747

This prospective study collected data on 31,766 infants born between 1993-2004 at a single institution. A total of 1339 children had genetic permissiveness/risk for celiac disease (HLA-DR, DQ genotypes listed in detail in the article) and were enrolled into 20-year prospective follow-up evaluation. The results showed that 5% of the population experienced a period of celiac disease autoimmunity (defined as elevated tTG for a period of 3 months). Some of these patients spontaneously resolve and, by age 15 years, 3% of patients will have celiac disease (defined as persistent tTG and at least Marsh 2 on biopsy).

The Association Between CMV Viremia or Endoscopic Features and Histopathological Characteristics of CMV Colitis in Patients with Underlying Ulcerative Colitis.
Yang H, Zhou W, Lv H, Wu D, Feng Y, Shu H, Jin M, Hu L, Wang Q, Wu D, Chen J, Qian J.
Inflamm Bowel Dis. 2017 May;23(5):814-821.
https://www.ncbi.nlm.nih.gov/pubmed/28426459

This study of 50 consecutive patients admitted for flare of ulcerative colitis with CMV found that half of the patients had concurrent CMV colitis. They observed statistically significant differences in the endoscopic findings between patients with CMV colitis and those without colon involvement, including the present of punched-out and irregular ulcers and cobblestone pattern. They also found a significantly higher number of CMV inclusions per high power field in patients with punched-out ulcers than in those without this feature, and suggest that targeted biopsies to these features may increase detection of CMV colitis.

Oligoclonal T-cell Receptor Repertoire in Colonic Biopsies of Patients with Microscopic Colitis and Ulcerative Colitis.
Günaltay S1, Repsilber D, Helenius G, Nyhlin N, Bohr J, Hultgren O, Hultgren Hörnquist E.
Inflamm Bowel Dis. 2017 Jun;23(6):932-945.
https://www.ncbi.nlm.nih.gov/pubmed/28498152

This study looked at regional diversity of the TCRβV-J region on T cells using next generation sequencing on colon biopsies from patients with collagenous colitis (n=26), lymphocytic colitis (n=15), ulcerative colitis (n=33), and controls (n=33), and found a lower evenness and richness ("a more oligoclonal character") in microscopic colitis patients compared to those with histologic remission, as well as in lymphocytic colitis compared with collagenous colitis. Higher diversity and richness was found in patient with ulcerative colitis and UC in remission. They suggest that the more oligoclonal TCRβ repertoire in lymphocytic colitis patients may be related to their more intermittent disease course.

Enteric Infection in Relapse of Inflammatory Bowel Disease: The Utility of Stool Microbial PCR Testing.
Axelrad JE, Joelson A, Nobel YR, Lawlor G, Green PHR, Lichtiger S, Lebwohl B.
Inflamm Bowel Dis. 2017 Jun;23(6):1034-1039.
https://www.ncbi.nlm.nih.gov/pubmed/28511200

This retrospective review of 214 IBD patients who underwent stool testing by PCR during an exacerbation of symptoms revealed that 26.8% had enteric infections. Of these, about half were due to Clostridium difficile, and half were due to enteric infections, most commonly E. coli species (47%). The gastrointestinal pathogen panel PCR used was a BioFire FilmArray, which tests for 22 pathogens, including bacteria, viruses, parasites, and bacterial toxins. Nine patients had more than one pathogen detected. Positive pathogen PCR testing was not significantly associated with any clinical, demographic, surgical, imaging, or therapy indicators.

The Mucosal Antibacterial Response Profile and Fecal Microbiota Composition Are Linked to the Disease Course in Patients with Newly Diagnosed Ulcerative Colitis.
Magnusson MK1, Strid H, Isaksson S, Simrén M, Öhman L.
Inflamm Bowel Dis. 2017 Jun;23(6):956-966.
https://www.ncbi.nlm.nih.gov/pubmed/28445247

This prospective study included forty-eight newly diagnosed UC patients, who were assessed for diseases severity and colonic disease extent over the course of 3 consecutive years based on the number and severity of disease flares. At the time of diagnosis and prior to starting any therapy, stool samples and intestinal biopsies were taken. Fecal microbiota analysis by the GA-map Dysbiosis test and gene expression profiling in the biopsies were able to discriminate between patients with mild and those with moderate/severe disease course. Expression at diagnosis of mucosal bactericidal/permeability-increasing protein was higher in the mild disease course group compared to the moderate/severe disease course group as confirmed on a larger cohort ($P = 0.0004$, $n = 44$) and was a statistically significant predictor of number of flares over the 3 years ($R = 0.395$, $P < 0.0001$).

Incidence, Risk Factors, and Outcomes of Colorectal Cancer in Patients With Ulcerative Colitis With Low-Grade Dysplasia: A Systematic Review and Meta-analysis.
Fumery M, Dulai PS, Gupta S, Prokop LJ, Ramamoorthy S, Sandborn WJ, Singh S.
Clin Gastroenterol Hepatol. 2017 May;15(5):665-674.e5.
https://www.ncbi.nlm.nih.gov/pubmed/27916678

This systematic literature review with meta-analysis was performed to evaluate the rate of development of advanced neoplasia and colorectal carcinoma (CRC) in ulcerative colitis (UC) patients found to have low-grade dysplasia (LGD). The authors note that similar analysis have been performed in the past but these omitted any analysis of risk factors associated with that progression. In the current review, 14 studies were selected which included 671 patients who underwent endoscopic surveillance following a diagnosis of LGD. A separate surgical cohort, extracted from 12 studies, consisted of 450 patients who underwent colectomy following a diagnosis of LGD. Similar to previous studies, the authors found that the pooled annual incidences of CRC and advanced neoplasia was 0.8% and 1.8% respectively. Analysis of risk factors for progression demonstrated that individuals whose LGD was diagnosed by expert gastrointestinal pathologists than by community pathologists had a higher risk of subsequent CRC. Additionally, the presence of concurrent primary sclerosing cholangitis, invisible dysplasia (dysplasia not appreciated endoscopically), distal location, and multifocal dysplasia were associated with higher rates of progression. There was no significant association with age, sex, or disease duration. Within the surgery cohort, CRC was identified in 17% of individuals with lower rates being appreciated in more recent studies compared to those performed prior to 2000. The authors discuss potential reasons for these findings and suggest these rates and risk factors should be considered when deciding on the follow-up strategy for UC patients diagnosed with LGD.

Microcarcinoid arising in patients with long-standing ulcerative colitis: histological analysis
Kanada S, Sugita A, Mikami T, Ohashi K, Hayashi H.
Hum Pathol. 2017 Jun;64:28-36.
https://www.ncbi.nlm.nih.gov/pubmed/28428105

This study reviewed 135 resections from patients with UC. 14 cases (10.4%) were found to have microcarcinoids (MC, defined in this study as macroscopically undetectable lesions with trabecular or nested architecture) which ranged from 0.1 to 5.5mm, all without distinct carcinoid tumors, vascular invasion, mitosis or necrosis. All MC were found in the rectum, showed neuroendocrine differentiation by IHC (positive for Chromogranin A, synaptophysin, NSE, N-CAM, and CK-LW), and were negative for CDX2. The MIB-1 index was approximately 1.5% for all lesions. More than half of cases showed multiple lesions, but the surrounding mucosa showed no increase in neuroendocrine cells. Half of patients with MC also had colitis associated cancer; while this may indicate that MC are associated with colitic cancer, the authors also point out that a greater number of sections are submitted in cases of colitic cancers, resulting in increased detection of MC. The authors conclude that because MC were not associated with carcinoids or aggressive features, these microscopic lesions may not represent true neoplasms.

Histopathologic Features of Colitis Due to Immunotherapy With Anti-PD-1 Antibodies.
Chen JH, Pezhouh MK, Lauwers GY, Masia R.
Am J Surg Pathol. 2017 May;41(5):643-654.
https://www.ncbi.nlm.nih.gov/pubmed/28296676

The authors of the study aimed to aid pathologists in recognizing the histopathologic features of colitis associated with programmed cell death protein 1 (PD-1) checkpoint inhibitor monotherapy. In their study, anti-PD-1 colitis was clinically diagnosed in 8 patients (3 Male 5 Female, age range 37-90 years, median age 63 years), based on the following criteria: (1) onset of diarrhea while on anti-PD-1 therapy for advanced stage malignancy, (2) clinical exclusion of other causes of diarrhea, (3) significant clinical improvement following withdrawal of anti-PD-1 therapy and administration of immunosuppressive therapy. The patients had been on anti-PD-1 therapy from 27 to 350 days before onset of grade 3 diarrhea in 7 patients and grade 1 in 1 patient. Other symptoms included abdominal pain/discomfort/cramping (5 patients) and cough (2 patients). Endoscopic colitis was noted in 5 patients (4 - pancolitis, 1- left sided colitis); in 1 case, the colitis was associated with pseudomembranes. One of two mutually exclusive patterns of mucosal injury was observed. The most common histopathologic pattern (5 of 8 patients) was active colitis with increased apoptosis, characterized by active inflammation, neutrophilic crypt microabscesses, increased crypt epithelial cell apoptosis, and presence of crypt atrophy/dropout. The remaining 3 patients showed lymphocytic colitis pattern with increased intraepithelial lymphocytes in surface epithelium, surface epithelial injury, and mononuclear expansion of the superficial lamina propria. Immunohistochemical stains for CMV were negative in all 8 patients. Features of chronicity were not identified in the initial biopsies

from these 8 patients. All 8 patients were withdrawn from anti-PD-1 therapy. After starting corticosteroids, all achieved response to therapy with 7 patients showing complete remission of diarrhea and 1 patient having substantial improvement. Two patients developed diarrhea months after anti-PD-1 therapy was stopped, and the repeat biopsies not only showed active colitis with apoptosis as the initial biopsies but also had features of chronicity in the form of basal lymphoplasmacytosis and mild crypt architectural irregularities, such as branching. In one patient with a clinical history of ulcerative colitis (UC), the biopsies at the time of suspected antiPD-1 colitis showed active colitis and no features of chronicity, responsive to infliximab and steroids. One month after restarting antiPD-1 therapy, the patient developed severe diarrhea with biopsies showing diffuse chronic active colitis, clinically recurrent UC, which may been precipitated by antiPD-1 therapy. Total colectomy revealed evidence of medically refractory UC. Based on these findings, the authors concluded that anti-PD-1 colitis most commonly presents as an active colitis with apoptosis and crypt atrophy/dropout and less commonly as lymphocytic colitis pattern. More importantly, features of IBD-type chronicity were observed in patients months after stopping anti-PD-1 therapy. This study showed that anti-PD-1 colitis has many features similar to anti-CTLA-4 colitis. Recognition and early diagnosis of anti-PD-1 colitis is important so that the checkpoint inhibitor can be stopped and immunosuppressive therapy can be instituted. Complex issues, including anti-PD-1 therapy in IBD patients and concurrent anti-CTLA4 therapy, require further investigation.

MCM2 expression in serrated polyps demonstrates aberrant cellular proliferation
Fortuna D, Boman B, O'Neill R, Palazzo J.
Hum Pathol. 2017 May;63:177-183.
https://www.ncbi.nlm.nih.gov/pubmed/28302537

This immunohistochemical study examined Ki-67 and MCM2 (a protein involved in DNA replication) expression among 16 microvesicular hyperplastic polyps, 58 SSA/P, 7 SSA with dysplasia, and 6 sections of normal colon. Normal colon showed MCM2 and Ki-67 staining limited to the lower ½ to 2/3 of crypts. By comparison, 81% of HP and 100% of SSA/P showed some crypts with full thickness MCM2 staining. SSA with dysplasia showed diffuse polyp staining, with additional aberrant staining in the adjacent normal tissues. The authors conclude that aberrant MCM2 staining in serrated polyps and adjacent mucosa indicates a field effect or microenvironment change that predisposes to malignancy.

IMP3 expression in biopsy specimens as a diagnostic biomarker for colorectal cancer
Wei Q, Zhou H, Zhong L, Shi L, Liu J, Yang Q, Zhao T.
Hum Pathol. 2017 Jun;64:137-144.
https://www.ncbi.nlm.nih.gov/pubmed/28412210

Previous studies have shown that IMP3 is expressed in CRC, is associated with lymph node metastasis, and is an independent prognostic biomarker. This retrospective study investigated the expression of IMP3 in hyperplastic polyps (HPs) and adenomas, and correlated results

between biopsy (n=300), polypectomy (n=633) and resection (n=395) specimens. IMP3 expression was seen in 0% normal tissue, 0% HPs, 2.8% adenomas, and 70.6% CRCs. Among the 300 biopsy specimens, 244 were originally diagnosed as CRC (and subsequently confirmed by resection), while the remaining 56 were called adenomas. Among these adenomas, resection showed 34 (60.7%) were in fact CRCs, and all stained for IMP3 on biopsy material. The authors show that all IMP3 positive biopsies in their study were ultimately CRC's upon resection. They conclude that IMP3 is a useful diagnostic biomarker for CRC by stratifying adenomas in biopsy diagnosis. The article suggests diagnosing CRC when an adenoma demonstrates IMP3 reactivity and provides a decision tree.

GNAS mutations are present in colorectal traditional serrated adenomas, serrated tubulovillous adenomas and serrated adenocarcinomas with adverse prognostic features
Liu C, McKeone DM, Walker NI, Bettington ML, Leggett BA, Whitehall VLJ.
Histopathology. 2017 Mar;70(7):1079-1088.
https://www.ncbi.nlm.nih.gov/pubmed/28164369

GNAS mutations were studied on a set of 50 TAs, 50 conventional TVAs (cTVAs), 43 SSAs, 196 TSAs, 56 serrated TVAs (sTVAs), and 459 CRCs from a special GI practice in Australia. GNAS mutations are present in 9.2% of TSAs, 7.1% of sTVAs, and 2.0% of CRCs. They were entirely absent in SSAs, TAs, and cTVAs. BRAF or KRAS mutation was seen in 77.4% of GNAS-mutant lesions, suggesting a relationship with the MAPK pathway which the authors suggest may drive the progression to CRC in select cases. In cancer cases, GNAS mutations were associated with mucinous features and serrated architecture.

Tumor size, tumor location, and antitumor inflammatory response are associated with lymph node size in colorectal cancer patients.
Rössler O, Betge J, Harbaum L, Mrak K, Tschmelitsch J, Langner C
Mod Pathol. 2017 Jun;30(6):897-904.
https://www.ncbi.nlm.nih.gov/pubmed/28233767

This study examines what factors might determine lymph node size in cases of colorectal carcinoma. The authors note that lymph node size is important in the pre-operative estimation of the presence of metastatic disease, which informs pre-operative management decisions including whether to give neo-adjuvant chemotherapy. While size of lymph nodes is used to identify likely metastatic tumor, other factors might influence lymph node size. This prospective cross-sectional study looked at 148 colorectal cancer cases with a mean of 28 lymph nodes per case and recorded the presence of metastatic tumor as well as associated tumor related parameters. After analysis, the authors found that lymph node size was related to the presence of metastatic disease, and the larger the node, the higher the risk of metastatic tumor. It was noted, however, that positive lymph nodes measuring ≤2 mm caused upstaging within the N category in one third of cases, but this did not change nodal status from negative to positive in these patients because all had positive larger nodes. Other tumor related factors

significantly associated with enlarged lymph nodes included large tumor size, right tumor location, and deep tumor penetration. Factors that did not have a statistically significant association with enlarged tumor size included microsatellite instability and lymphocytic antitumor reaction.

Neoadjuvant therapy in microsatellite-stable colorectal carcinoma induces concomitant loss of MSH6 and Ki-67 expression
Kuan SF, Ren B, Brand R, Dudley B, Pai RK.
Hum Pathol. 2017 May;63:33-39.
https://www.ncbi.nlm.nih.gov/pubmed/28232158

These authors aimed to provide a mechanism for the observed loss of MSH6 and Ki-67 IHC expression in post-neoadjuvant microsatellite-stable (MSS) colorectal carcinomas (CRCs). The IHC pattern of MSH2, MSH6, and Ki-67 in 114 MSS CRC was compared with (n=50) and without (n=64) preoperative neoadjuvant therapy. This was compared to 3 Lynch syndrome associated CRCs with confirmed MSH6 germline mutation.

The authors found that MSH6 and Ki-67 IHC loss of expression was closely linked in the sporadic setting. For example, loss of MSH6 and loss of Ki-67 were both found in the same tumor areas. Although post-neoadjuvant tumors were more likely to show a greater degree of IHC loss (with some cases showing complete loss of staining), both pre- and post-neoadjuvant MSS tumors showed this tandem loss of MSH6 and Ki-67. By comparison, MSH6 deficient LS CRC showed complete loss of MSH6 with retention of Ki-67 expression.

The authors postulate that loss of MSH6 and Ki-67 expression in MSS CRC may be an indicator of therapeutic effect and better patient survival, although this study lacked sufficient follow-up to draw any conclusions.
Based on their findings, the authors recommend:

1. MMR IHC on *pre*-neoadjuvant biopsies, and/or
2. PCR MSI for *post*-neoadjuvant samples, and/or
3. IHC on *post*-neoadjuvant samples includes both MSH6 and Ki-67 with tandem interpretation:
    a. MSH6 loss and Ki67 loss: proficient MSH6 status
    b. MSH6 loss and Ki-67 retained: deficient MSH6 status and concern for Lynch Syndrome

Tailored Treatment Strategy for Locally Advanced Rectal Carcinoma Based on the Tumor Response to Induction Chemotherapy: Preliminary Results of the French Phase II Multicenter GRECCAR4 Trial

Rouanet P, Rullier E, Lelong B, Maingon P, Tuech J, Pezet D, Castan F, Nougaret S, and the GRECCAR Study Group

Dis Colon Rectum 2017; 60: 653–663

https://www.ncbi.nlm.nih.gov/pubmed/28594714

This multicenter randomized trial study investigated the possibility of tailoring preoperative chemoradiation (CRT) to the response to FOLFIRINOX induction chemotherapy in patients with locally advanced rectal cancer (LARC). Current standard of care therapy for LARC is neoadjuvant CRT followed by surgery, which has had no improvement on overall survival, but has decreased local recurrence rates to less than 10%. Some patients have a good response to CRT but others have a poor response. Therefore, the authors postulate an individualized tailored approach to treatment to avoid over treating the good responders, avoid adverse outcomes (such as sexual dysfunction, infertility and bone marrow depletion), and discover a more effective approach for the poor responders. Therefore, this study sought to determine whether induction chemotherapy could be used as a tool to identify subgroups of patients where radiation protocols could be deescalated or intensified to reach a minimum of 90% R0 resection rates in all arms. Favorable responders, based on MRI evidence of tumor shrinkage, were randomized to either arm A (experimental/immediate surgery) or arm B (standard) and the unfavorable group were randomly assigned to arm C (standard) or arm D (experimental/intensified CRT). Of the 206 patients enrolled in the study 194 were evaluated by MRI. 15% were good responders and 85% were poor responders. 133 patients were ultimately analyzed based on the treatment they received and 89% were predicted to have a positive radial margin. Overall, the results showed R0 resection rates of 100% (10 of 10 patients; 90% CI: 74–100) in arm A, 100% (19 of 19 patients; 90% CI: 85–100) in arm B, 83% (34 of 40 patients; 90% CI: 72–91) in arm C, and 88% (34 of 40 patients; 90% CI: 77–95) in arm D. CRMs >1mm were 0%, 0%, 12%, and 5% in arms A, B, C, and D, respectively. The authors state that overall the results for the chemosensitive patients (arm A/deescalation) who received immediate surgery were favorable although no formal conclusions could be drawn. For the patients with chemoresistant tumors (arms C and D), results from the patients who received CRT 'intensification' (arm D), are 'sufficiently promising' to consider moving to a larger trial.

High expression of P2X7R is an independent postoperative indicator of poor prognosis in colorectal cancer

Qian F, Xiao J, Hu B, Sun N, Yin W, Zhu J.

Hum Pathol. 2017 Jun;64:61-68.

https://www.ncbi.nlm.nih.gov/pubmed/28412208

P2X7R is an ATP sensing receptor highly expressed in immune cells, is heavily implicated in inflammation and inflammation-related diseases, and has been implicated in some tumors. The authors investigate P2X7R in 12 CRCs by Western blot and 116 CRCs by IHC, compare this to

normal tissues, and correlate with clinicopathologic features and survival. Western blot showed P2X7R upregulated in CRC as compared to paired noncancerous tissues, and IHC showed homogeneous nuclear and cytoplasmic reactivity in tumor cells. Expression highly correlated with larger tumor size, lymph node metastasis, higher TNM stage, higher tumor grade, and worse prognosis. The authors discuss that P2X7R may activate Akt and NF-kB p65 pathways and drive CRC proliferation and suggest that P2X7R might be an ideal molecular target for colorectal cancer.

Mutational signature analysis identifies MUTYH deficiency in colorectal cancers and adrenocortical carcinomas.
Pilati C, Shinde J, Alexandrov LB, Assié G, André T, Hélias-Rodzewicz Z, Ducoudray R, Le Corre D, Zucman-Rossi J, Emile JF, Bertherat J, Letouzé E, Laurent-Puig P.
J Pathol. 2017 May;242(1):10-15.
https://www.ncbi.nlm.nih.gov/pubmed/28127763

The authors of this study used whole exome sequencing and analysis of previously published mutational profiles of colorectal lesions to investigate the relationship between *MUTYH* defects and particular mutational signatures. MUTYH is a DNA glycosylase involved in the base excision repair pathway which corrects for oxidative damage of DNA. Sequencing was performed of 37 advanced colorectal carcinomas, and multiple lesions from a *MUTYH* associated polyposis patient including two adenomas and one carcinoma. The mutational profiles of these lesions were analyzed for specific mutational signatures as reported in the COSMIC database. Three distinct signatures were identified including signatures 1, 5, and 18. Of these, the etiology of mutational signature 18, characterized by enrichment of C>A transversions, had previously been unknown. Analysis of the material from the *MUTYH* associated polyposis patient not surprisingly demonstrated germline mutations in *MUTYH*, but also mutational signature 18 in both the invasive tumor as well as the adenomas. Of the 37 other carcinomas analyzed, the two tumors which demonstrated mutational signature 18 also harbored germline mutations in *MUTYH*. The authors went on to investigate other publicly available mutational profiles of colorectal adenocarcinomas, such as the TCGA data, and again found an association of mutational signature 18 with *MUTYH* mutations. The authors suggest that mutational signature 18 reflects the accumulation of C>A mutations that may accumulate in the context of defective base excision repair. They also note that while this signature in colorectal carcinomas appears to suggest mutations in *MUTYH*, other malignancies such as breast carcinoma and neuroblastoma, which demonstrate increased incidence of mutational signature 18, must do so by other mechanisms as they do not demonstrate alterations in this gene. Finally, the authors note that mutational signatures may be a useful tool in the context of genetic counselling and treatment.

Recommendations on Surveillance and Management of Biallelic Mismatch Repair Deficiency (BMMRD) Syndrome: A Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer

Durno C, Boland CR, Cohen S, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, Levin TR, Lieberman D, Robertson DJ, Rex DK.
Gastroenterology. 2017 May;152(6):1605-1614.
https://www.ncbi.nlm.nih.gov/pubmed/28363489

Guidelines for Biallelic Mismatch Repair Deficiency (BMMRD) syndrome are provided in this article. Pathologists may aid in detecting these patients:

Clinical and Laboratory Features to Raise Suspicion for Possible BMMRD

1. Child or young adult with a Lynch syndrome cancer (colorectal, small bowel, ureter, endometrial, etc)
2. Child or young adult with colonic adenomatous polyposis not explained by a known polyposis syndrome mutation (e.g., familial adenomatous polyposis, MUTYH-associated polyposis)
3. Any child or young adult with cancer plus parental consanguinity, café-au-lait macules, or features of neurofibromatosis, not explained by other confirmed germline mutation (ie, neurofibromatosis)
4. Any cancer with abnormal immunohistochemistry for DNA-MMR proteins in normal and tumor tissue
5. History of brain cancer, lymphoma, or leukemia without history of radiation
6. Any child or adult with hypermutated tumor

mTOR activity and its prognostic significance in human colorectal carcinoma depending on C1 and C2 complex-related protein expression

Sticz T, Molnár A, Márk A, Hajdu M, Nagy N, Végso G, Micsik T, Kopper L, Sebestyén A.
Sticz T, *et al*.
J Clin Pathol 2017;70:410–416.
https://www.ncbi.nlm.nih.gov/pubmed/27729429

This retrospective study utilizes immunohistochemistry to evaluate mTORC1 and C2 activity in colorectal tumors with the aim to provide information about prognosis and guide therapeutic decisions. mTOR (mammalian/mechanistic target of rapamycin) is a serine/threonine protein kinase that exists in two complexes mTORC1 (which includes Raptor) and mTORC2 (which includes Rictor). Current mTOR inhibtors directly target mTORC1 but not mTORC2, although there may be secondary inhibition. A variety of somatic and germline mutations within the mTOR pathway have been discovered in a variety of neoplasms. However, the effectiveness of different inhibitors in several types of solid tumors is still being investigated in clinical trials. This study analyzed conventionally treated colorectal carcinoma for mTORC1 and mTORC2 activity and correlated with clinical data. 103 patients with colorectal adenocarcinoma (72 colon, 31 rectum) were studied. Tissue microarrays were made and IHC for mTORC,

phosphorylated-mTOR, Raptor, Rictor, phosphorylated-S6 and phosphorylated-4EBP1 and phosphorylated-AMPK. 76 cases (73.8%) showed high mTOR activity with the co-expression of p-S6, p-4EBP1 and p-mTOR as the most reliable markers of mTOR activity. Patient survival data and IHC results showed that low mTOR activity correlated significantly with good prognosis. Additionally, those cases showing dominant Rictor expression had the longest 5 year overall survival. These results are similar to previously reported studies, although the authors state that this study confirms stage and grade independent activity of mTOR signaling in colorectal adenocarcinoma.

Dual Immunostain With SATB2 and CK20 Differentiates Appendiceal Mucinous Neoplasms From Ovarian Mucinous Neoplasms

Li Z, Roth R, Rock JB, Lehman A, Marsh WL, Suarez A, Frankel WL

Am J Clin Pathol 2017 147 (5): 484-491.

https://www.ncbi.nlm.nih.gov/pubmed/28340228

This study looks at the problem of identifying the site of origin of mucinous neoplasms in the peritoneal/pelvic cavities, where appendiceal mucinous and ovarian mucinous neoplasms are major considerations. With this problem in mind, the authors look at the expression of Special AT-rich sequence binding protein (SATB2), CDX2, CK20, and villin by immunohistochemistry using dual stains that include a nuclear stain (SATB2 or CDX2) coupled with a cytoplasmic stain (CK20 or villin), and explore the clinical utility of these combinations in determining the tumor's primary site of origin. The study set included 40 appendiceal and 18 ovarian mucinous neoplasms and the tumors were evaluated by immunohistochemistry on tissue microarray. Sensitivities and specificities of both the single stain results and dual stain results were calculated and the double stain combination of SATB2 and CK20, when both were positive, was found to have the best potential clinical utility for identifying appendiceal origin (sensitivity of 80% and specificity of 100%). The authors note that their study was somewhat limited by small cohort size and use of TMA (small sampling of tumor).

Heterotopic Pancreas of the Gastrointestinal Tract and Associated Precursor and Cancerous Lesions: Systematic Pathologic Studies of 165 Cases.

Jun SY, Son D, Kim MJ, Kim SJ, An S, Park YS, Park SR, Choi KD, Jung HY, Kim SC, Yook JH, Kim BS, Hong SM.

Am J Surg Pathol. 2017 Jun;41(6):833-848.

https://www.ncbi.nlm.nih.gov/pubmed/28368927

The authors of this study conducted a systematic review of the clinicopathologic findings of resected heterotopic pancreas (HP) in 165 patients (mean age 52.2±15.3 years; M:F-1.4; mean size 14±8.5 mm) and their association with acinar ductal metaplasia (ADM), pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasms (IPMN), and adenocarcinoma. HP has a prevalence of 0.3% to 13.7% in the general population and may be symptomatic or incidentally detected during GIT tumor resections. According to the Heinrich

classification, 109 HPs (66%) were type 1 (all three components acini, ducts and islets); 45 (27%), were type 2 (acini and ducts); and 5 (3.0%) were type 3 (only ducts). Six cases (4%) were unclassifiable (4 contained acini and islets and 2 only acini). Locations were as follows: 57 (35%) gastric, 56 (34%) duodenal, 30 (18%) omental, and 22 (13%) jejunal. Of the 135 GI tract HP cases, HP involved the muscularis propria or subserosa in 89 cases (66%); and 46 (34 %) had mucosal or submucosal involvement. Eighty-six cases (52.1%) were incidentally found in resected specimen for other GI tract tumors, commonly gastric adenocarcinoma (55 cases) and periampullary carcinoma (29 cases). The remaining 79 cases (47.9%) showed various GI symptoms. Symptomatic HPs were larger (P=0.05), more common in younger patients and in a gastric location (both P<0.001), and more frequently associated with lymphoid cuffs (P=0.03) than incidentally found HPs. PanIN was found in 68 cases (PanIN1- 58, PanIN2 – 9, PanIN3 - 1) and low-grade IPMN in 2 cases. There was no case of adenocarcinoma arising in HPs. ADMs were present in 123 cases and, in 63 cases, were adjacent to PanIN or IPMN. When compared to duodenal HPs, gastric/jejunal were more frequently symptomatic (P<0.001), deeply located (P=0.03), and associated with lymphoid cuffs (P=0.008) and PanIN/IPMN (P=0.001) than duodenal HPs. The GI tract HPs were commonly associated with ADM (117/135) and PanIN/IPMN (68/135); those with PanINs/IPMNs were larger (P<0.001), more frequently located in the stomach (P=0.001), had deeper wall involvement (P=0.03), and more often showed infiltrative growth (P<0.001) and lymphoid cuffs (P=0.02). Four HPs containing PanINs (1 case PanIN3) abutted gastric adenocarcinomas; all 4 adenocarcinomas did not show features of pancreatic ductal adenocarcinoma and were wild-type KRAS with intact SMAD4/DPC4 expression suggesting synchronous lesions rather than adenocarcinoma arising from HP. Based on these findings the authors concluded that symptomatic HP is associated with younger age, larger size, gastric location, and lymphoid cuffs. HPs containing PanINs/IPMNs (usually low grade) are larger and more common in the stomach, have deeper wall location, show infiltrative growth and lymphoid cuffs, and, when associated with adenocarcinomas, those adenocarcinomas do not have features of pancreatic ductal adenocarcinoma.

Recent Discoveries in the Genetics of Familial Colorectal Cancer and Polyposis.
Valle L.
Clin Gastroenterol Hepatol. 2017 Jun;15(6):809-819.
https://www.ncbi.nlm.nih.gov/pubmed/27712984

This paper provides a thorough review discussing some recently recognized genetic alterations thought to contribute to cases of hereditary colorectal cancer syndromes. While impossible to adequately cover in this short summary, the author discusses how the advent of massively parallel sequencing-based approaches and genome-wide copy number techniques have identified a number of additional hereditary cancer genes. In brief, novel hereditary colorectal cancer genes *POLE* and *POLD1* are discussed in the context of autosomal dominant attenuated adenomatous polyposis. *NTHL1*-associated adenomatous polyposis, a recessive adenomatous polyposis syndrome similar to *MUTYH*-adenomatous polyposis is also summarized. The discovery of *GREM1* alterations in cases of mixed polyposis syndrome, which can present with an unusual array of serrated polyps, Peutz-Jeghers polyps, juvenile polyps, and conventional

adenomas, are also presented. Other topics include *RNF43*-associated serrated polyposis, *AXIN2* related polyposis, and constitutional mismatch repair deficiency (CMMRD) childhood cancer predisposition syndrome. The author notes that screening for several of these genes has already been implemented in routine genetic testing and others are sure to follow as our understanding of them matures.

Genetic analyses of isolated high-grade pancreatic intraepithelial neoplasia (HG-PanIN) reveal paucity of alterations in TP53 and SMAD4.
Hosoda W, Chianchiano P, Griffin JF, Pittman ME, Brosens LA, Noë M, Yu J, Shindo K, Suenaga M, Rezaee N, Yonescu R, Ning Y, Albores-Saavedra J, Yoshizawa N, Harada K, Yoshizawa A, Hanada K, Yonehara S, Shimizu M, Uehara T, Samra JS, Gill AJ, Wolfgang CL2, Goggins MG, Hruban RH, Wood LD.
J Pathol. 2017 May;242(1):16-23.
https://www.ncbi.nlm.nih.gov/pubmed/28188630

The authors of this multicenter study aimed to investigate the molecular characteristics of isolated high-grade pancreatic intraepithelial neoplasia (HG-PanIN) as well as the immunohistochemical expression of SMAD4 and p53 in these lesions. The authors note that most prior studies of HG-PanIN have utilized lesions that were present in specimens containing concurrent invasive pancreatic adenocarcinomas. As it is well established that pancreatic adenocarcinomas can extend into pre-existing ducts through 'cancerization of the ducts', these previous studies may have been examining foci of intraductal spread. In the current study, a cohort of 23 isolated HG-PanINs from 21 patients were examined by various methodologies including whole-exome sequencing, targeted next generation sequencing (NGS), and immunohistochemistry. The most frequently mutated gene was *KRAS* (16 of 17 tested cases; 94%) while alterations in *RNF43*, *CDKN2A*, *GNAS*, *TP53*, *PIK3CA*, *TGFBR2*, and *ARID1A* were noted in a minority of cases. A number of cases also harbored low-grade PanIN (LG-PanIN) which were found to not only be largely molecularly independent of the concurrent HG-PanIN, but also independent of one another in cases containing multiple foci. No significant alterations in *SMAD4* were identified by NGS or exome sequencing of a limited set of the cohort. While immunohistochemical studies demonstrated aberrant expression of p53 in 3 of 16 cases tested, only one of these lesions was shown to have a somatic *TP53* mutation. *SMAD4* expression was retained in all 17 cases examined. In their discussion, the authors note their significant absence of *SMAD4* mutations and limited *TP53* alterations is in stark contrast to previous studies that suggested these changes were frequent in HG-PanIN. They postulate that these previous studies may have in fact been examining intraductal involvement of an invasive carcinoma or that perhaps isolated HG-PanIN is biologically different from HG-PanIN associated with invasive carcinoma. Finally, they suggest that inactivation of *TP53* and *SMAD4* are late alterations in pancreatic neoplasia development and are largely limited to invasive carcinoma

Mutation Profile and Fluorescence In Situ Hybridization Analyses Increase Detection of Malignancies in Biliary Strictures.

Gonda TA, Viterbo D, Gausman V, Kipp C, Sethi A, Poneros JM, Gress F, Park T, Khan A, Jackson SA, Blauvelt M, Toney N, Finkelstein SD.

Clin Gastroenterol Hepatol. 2017 Jun;15(6):913-919.e1.
https://www.ncbi.nlm.nih.gov/pubmed/28017843

This prospective industry sponsored study was performed to evaluate the role PCR-based DNA analysis may play in the diagnosis of biliary strictures. They compared the diagnostic accuracy of conventional cytology, FISH, and mutational profiling of supernatant fluid free DNA from a cohort of 100 patients treated over a period of two years for biliary strictures. Patients with an obvious mass on endoscopic ultrasound or a positive rapid on-site evaluation of cytology were excluded. All specimens included were bile duct brushings and the mutational analysis included evaluation of point mutations in *KRAS* and loss of heterozygosity mutations in a number of tumor suppressor genes. As far as outcomes, the delineation of malignant versus benign strictures were based on subsequent follow-up studies including pathologic examinations and repeat imaging at least 12 months following the initial bile duct brushing. Of the included patients, 41% had malignant strictures while 59% had nonmalignant strictures. Routine cytology had a specificity of 100% with a sensitivity of 32% when all strictly “positive” results were considered diagnostic of malignancy. This sensitivity improved to 44% when diagnoses of “suspicious” were also considered malignant, without affecting the specificity. While the individual sensitivities of these tests were below that of routine cytology, FISH and mutational profiling identified an additional 9 and 8 malignancies not detected by cytology, respectively. By considering a positive result from any of the three modalities as diagnostic of malignancy the sensitivity rose to 73% while the specificity remained 100%. The authors state that their results support the use of both FISH testing and mutational profiling in the evaluation of cytology-negative or indeterminate biliary strictures. They also note that both testing procedures can be performed on specimens acquired in current clinical practice and would not require additional procedure time or expertise.

Journals Reviewed (May & June 2017)
Histopathology
Archives of Pathology and Lab Medicine
Modern Pathology
American Journal of Clinical Pathology
Journal of Pathology
Journal of Clinical Pathology
American Journal of Pathology
Human Pathology
Cancer Cytopathology
American Journal of Surgical Pathology
Advances in Anatomic Pathology
Journal of Molecular Diagnostics
Gastrointestinal Endoscopy
Gastroenterology
Gut
American Journal of Gastroenterology
Clinical Gastroenterology Hepatology
Inflammatory Bowel Diseases
Diseases of the Colon and Rectum

Thank you to our Reviewers:

Ashwin Esnakula, MD; University of Florida Gainsville
Dora Lam-Himlin, MD; Mayo Clinic Arizona
Ilyssa Gordon, MD, PhD; Cleveland Clinic
Michael Feely, DO; University of Florida Gainsville
Nora Joseph, MD; NorthShore University HealthSystem
Raga Ramachandran, MD, PhD; University of California San Francisco
Steve Hart, MD; University of California Los Angeles