Introduction: Transition (TZ) and normal zone (GZ) in HD pull-through specimens show variable density of calretinin-positive mucosal nerve fibers. This variability may also be present in GZ in non-HD. Despite the variability, IPA may aid in defining a cutoff that separates the aganglionic segment (AZ) in HD from the GZ in non-HD.

Methods: Rectal biopsies from HD and non-HD and HD pull-through specimens were retrieved (2010–2017), and calretinin immunohistochemistry was performed. The immunostained slides were scanned and multiple images were captured (×100, JPEG format). Pixel count (PC), defined as the percentage of calretinin-stained pixels in the mucosa calculated by IPA as previously described, was measured for each image. Pearson’s correlation coefficient was calculated between the PC and the location of the biopsies.

Results: In the non-HD group, 62 biopsies taken at 0 to 4 cm (median 2 cm) from the dentate line were collected from 28 patients (age 4 days to 273 months, median 37 months), and 243 images (2–10 per biopsy, mean 3.5 images/case) were captured. In the HD group, 46 biopsies/segments were collected from 13 patients (age 2 days to 88 months, median 0.5 months) and 110 images (2–13 images/case, mean 2.4 images/case) were captured. Average PC was 0.482% in non-HD and 0.0153% in HD group, respectively (P < .0001). The average coefficient of variation in the non-HD group was 0.45. No correlation was found between the PC and the location of the biopsies. All (100%) non-HD PCs were >0.06%, and 45 of 46 (98%) HD PCs were <0.06%.

Conclusion: Although PC varies along the distance in non-HD patients, PC in non-HD is almost always higher than AZ in HD. Thus, defining a cutoff by IPA would aid in HD diagnosis. Defining the TZ in HD remains a challenge given the variation of the PC even in non-HD biopsies. Further study is warranted.

Using FOXP3 and CD25 Double Staining to Identify Regulatory T Cells in Tissue Microarrays

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Objectives: Regulatory T cells (Tregs) have been proposed to dampen functions of antineoplastic immune cells and thus promote cancer progression. They express CD25 and transcription factor Forkhead box P3 (FOXP3). The method to identify Tregs with CD25/FOXP3 double staining in the literature was mainly by flow cytometry analysis, which is not practical in daily practice. Here, we investigate the possibility of using FOXP3/CD25 double immunohistochemical staining to identify Tregs in tumor tissues.

Methods: Tissue microarrays (TMAs) were manually built by utilizing tumor tissues from 26 colons, 19 kidneys, 16 ovaries, 15 endometria, 14 breasts, 9 lungs, 5 head and neck tissues, 6 soft tissues, and 14 others. Tonsillar tissue was used as positive control. Double immunohistochemical staining for FOXP3/CD25 was performed by using cocktail antibodies of rabbit anti-human CD25 and mouse anti-human FOXP3. Detection was performed by using double secondary antibody. The FOXP3/CD25 double-positive cells were microscopically evaluated.

Results: The FOXP3/CD25 double immunohistochemical stain highlighted the FOXP3+/CD25high Tregs in control tonsillar tissue and a variety of tumor tissues in TMAs. Both intratumoral and intrastromal Tregs were identified. The amounts of FOXP3+/CD25high Tregs vary in different tumor types and organs of interest. For example, pulmonary squamous cell carcinomas generally show prominent Tregs, and renal cell carcinomas show minimal Tregs. Ovarian high-grade serous carcinomas show significantly more Tregs than borderline tumors. The amount of Tregs is variable in individual colon cancer.

Conclusion: Tregs in tumor tissues can be evaluated by utilizing double FOXP3/CD25 staining in daily practice. One of the advantages of the immunohistochemical stain over flow cytometry is being able to evaluate the location of Tregs, for instance, intratumoral or intrastromal. The high frequency of Tregs in pulmonary squamous cell carcinoma and ovarian high-grade serous carcinoma can potentially be targeted by using immunotherapy.

Apocrine Carcinoma of the Breast: A Clinicopathological Review of Five Cases With Histological and Immunohistochemical Evidence of EMT

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Objectives: Apocrine carcinoma is a carcinoma showing cytological and immunohistochemical features of apocrine cells in >90% of the tumor cells (WHO 2003). Apocrine carcinomas are typically GCDFP-15 positive. Recently, they have been shown to be ER, PR negative but AR positive. Some pathologists have argued that it should be considered a subtype of breast carcinoma because of its unique microscopic appearance. We evaluate five cases of apocrine carcinoma, including some evidence of epithelial mesenchymal transition.

Methods: A 2-year prospective study of formalin-fixed, paraffin-embedded tissue of all breast lesions diagnosed as apocrine carcinoma was retrieved. H&E slides were cut and stained. Immunohistochemical stains were done using six antibodies, ER, Her2, PR, AR, E-cadherin, and...
vimentin, using the DB-Biotech protocol. Data were analyzed using SPSS version 20.

**Results:** Five cases of apocrine carcinoma were reviewed. Age range was 25 to 60 years with a mean age of 43.6 ± 14.0. Histology showed tumor nests having hyperchromatic nuclei, prominent nucleoli, and granular cytoplasm; some spindling of tumor cells was seen. ER and PR were negative, Her2 was positive in three cases, and AR was positive in all tumors. Two cases showed loss of E-cadherin and focal areas of positive staining with vimentin.

**Conclusion:** Apocrine breast carcinoma is a distinct type of breast cancer that may signify a bad prognosis in our environment because of its presentation at younger age group and tendency for it to display EMT. We advocate molecular testing for possible targeted therapy in the future.

**IgG Immunophenotype in a Case of Idiopathic Giant Cell Myocarditis**

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**Objective:** Idiopathic giant cell myocarditis (IGCM) is a rare, rapidly progressive form of myocarditis with poorly understood etiology and associated with fulminant heart failure, refractory ventricular arrhythmias, and conduction system abnormalities. Previous reports have associated IGCM with autoimmune diseases. Response to rituximab in individual cases also suggests this possibility. Recently, several autoimmune entities have been subclassified into IgG4 and non-IgG4 subtypes. We present a case of IGCM where the expression of IgG and IgG4 was evaluated.

**Methods:** Our patient is a 71-year-old female with a medical history of hypertension, hyperlipidemia, and osteoarthritis. She initially presented with syncope due to heart block and then developed dyspnea. Cardiac catheterization showed elevated central venous filling pressures and a low cardiac output state. A persistently elevated troponin was documented. A pacemaker was implanted and endomyocardial biopsies were performed. The patient presented normalization of troponin levels with high-dose steroids.

**Results:** Histologic sections revealed five endomyocardial fragments. Two consisted entirely of fibrous tissue and granulation tissue with a mononuclear inflammatory infiltrate. The remaining biopsy fragments revealed myocardium being replaced by extensive multifocal infiltrates of lymphocytes and plasma cells. Multinucleated giant cells and histiocytes (CD68+) were also present. Other causes of granulomatous myocarditis, including tuberculosis, parasites, viruses, fungi, and sarcoidosis, were excluded. Overexpression of IgG and lack of expression of IgG4 was identified.

**Conclusion:** Autoimmune mechanisms have been proposed in IGCM. The identification of IgG overexpression with absence of IgG4 in our case supports the hypothesis of IGCM as an autoimmune entity that is not IgG4 related. This finding suggests that the pathogenesis of IGCM is associated with a mechanism involving overproduction of IgG1 to 3.

**Preoperative Diagnosis of Pulmonary Adenosquamous Carcinoma: Practice Patterns and Challenges**

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**Introduction:** Pulmonary adenosquamous carcinoma (PASC) is a rare histopathologic entity that presents unique diagnostic challenges in preoperative sampling. A tumor must only have 10% of both glandular and squamous differentiation to meet the World Health Organization definition for adenosquamous carcinoma; therefore, a small biopsy or cytology specimen is unlikely to be diagnostic of PASC in tumors that heavily favor one morphology. Since there are few reported successes in preoperative diagnosis of PASC, we sought to examine practice patterns to determine whether preoperative diagnosis of this entity could be improved.

**Methods:** We retrospectively examined 30 cases of PASC diagnosed on resection at our medical center between 2008 and 2017.

**Results:** Fifteen cases (50%) were sampled preoperatively with examination of cytologic or surgical specimens, including bronchoalveolar lavage, bronchial brushing, fine-needle aspiration (FNA), and/or biopsy. Ten cases had both preoperative cytology and biopsy performed; eight of them were positive for malignancy, with three cases diagnosed as squamous cell carcinoma, two cases diagnosed as adenocarcinoma, and two cases diagnosed as poorly differentiated carcinoma. In one case, the biopsy specimen was read as adenocarcinoma with squamous differentiation. In examining the immunohistochemistry performed on these cases, only three had both TTF-1 and p63 performed, and one case had both TTF-1 and p40 performed.

**Conclusion:** In our cohort, only one case was diagnosed with features suggestive of PASC on preoperative sampling. This highlights the limited ability of cytology or biopsy to fully represent a neoplasm with multiple morphologies. Given PASC represents less than 5% of primary lung neoplasms, there may be observer bias toward the most common non–small cell lung cancers, adenocarcinoma and squamous cell carcinoma. The inconsistent pattern of immunohistochemical stain use suggests that