PULMONARY PATHOLOGY JOURNAL CLUB
(February 2020 Articles)

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March 30, 2020

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1. Sholl LM. Programmed death ligand 1 immunohistochemistry: can we agree on this? Histopathology 2020; 76:189-190.
**Articles for Discussion**

**The Role of Histologic Grading and Ki-67 Index in Predicting Outcomes in Pulmonary Carcinoid Tumors**
Dermawan JT, Farver CF.

**Purpose:** To (1) examine the correlation between histologic type (typical vs atypical), Ki-67 proliferation index, major morphologic pattern, and lymphovascular invasion status with respect to outcome in terms of tumor recurrence, and to (2) propose a modified staging system for pulmonary carcinoid tumors

**Methods:**
- All typical and atypical pulmonary carcinoids from one institution (1995-2016) were reviewed
- The following information was collected for each case: tumor size, nodal status, histologic type, mitotic count, morphologic pattern [Figure 2], recurrence, and overall survival
- Patients with multiple malignancies, patients without at least 3 months follow-up and repeat chest CT, cases with positive margins, and cases without complete data were excluded
- FFPE tissue for each case was stained with Ki-67; slides were digitalized at maximum resolution
- At least 4 hotspots (highest Ki-67 staining) were manually selected for each; Ki-67 index was calculated using a digital algorithm (percent positive nuclei, staining intensity, etc) [Figure 1]
- A minimum of 1,000 nuclei was analyzed for each slide (average 22,000 nuclei/case)

**Results:**
- 176 pulmonary carcinoid tumors with complete data [Table 1]; 13 cases (7%) experienced recurrence (5 AC and 8 TC); only 4 patients (2%) died of metastatic carcinoid tumor
- The mean Ki-67 index was significantly higher in AC compared to TC, increased with higher TNM stage, and was higher in tumors which recurred compared to those that did not [Table 2]
- There were no significant differences for mean Ki-67 index with respect to morphologic pattern
For all carcinoids, histologic type (TC vs AC) and Ki-67 were significant predictors of recurrence on univariate analysis, but only Ki-67 was significant on multivariate analysis [Table 3].

For TC alone, only Ki-67 was a significant predictor of recurrence (for both UV and MV) [Table 4].

The optimal cutoff for Ki-67 to predict recurrence in TC was 5% (determined by ROC) [Figure 3].

Fig 4A shows that carcinoids with Ki-67>5% significantly more likely to recur than with Ki-67≤5%.

The authors separated cases into 3-tiers based on histologic type and Ki-67 index:
- **Grade 1**: TC with Ki-67 index ≤5%,
- **Grade 2**: TC with Ki-67 index >5%,
- **Grade 3**: AC (regardless of Ki-67 index).

Fig 4B shows a significantly increased likelihood of recurrence with each increase in grade.

The authors propose a modified staging scheme to predict recurrence based on this 3-tier system: keep original TNM stage for Grade 1 tumors, upstage Grade 2 tumors to at least stage II, and upstage Grade 3 tumors to at least stage III [Table 5].

Fig 5 shows that this system correlated better with recurrence compared to current AJCC system.

**Take Home Messages:**
- The major value of a 3-tier grading system that incorporates Ki-67 index is that it risk stratifies TC into cases with low likelihood vs cases with intermediate likelihood of recurrence.
- The proposed modified staging correlates well with risk of recurrence and is easy to implement.
- But reliable calculation of Ki-67 is a challenge (for both automated and manual quantification).

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**Background:** Highly aggressive thoracic neoplasms characterized by SMARCA4 deficiency and undiff round cell or rhabdoid morphology have recently been described and are proposed to represent thoracic sarcomas. However, it is unclear whether these tumors might actually be sarcomatoid carcinomas.
Purpose: Define the clinicopathologic, immunohistochemical, and genomic characteristics of SMARCA4 (BRG1) deficient thoracic sarcomatoid tumors (SD-TSTs) with round cell and/or rhabdoid morphology and compare to SMARCA4 deficient conventional NSCC in attempt to define the nosologic relationship of these two sets of SMARCA4 deficient tumors in the lung.

Methods:

Study Design
- Retrospective review of tumors from MSKCC archives

Sample Selection
- 22 SMARCA4-deficient thoracic sarcomatoid tumors (SD-TSTs) were identified from the pathology service at Memorial Sloan Kettering Cancer Center as follows:
  - 9 cases prospectively during the period of 2016-2017
  - 8 cases were identified by a retrospective re-review of thoracic tumors harboring SMARCA4 truncating mutations or deletions in the cBioPortal database of MSK-IMPACT next-generation sequencing (NGS) results.
  - 5 cases were identified by a retrospective search of pathology database of unclassified undifferentiated thoracic tumors in patients below 40 years or whose pathology mentioned “rhabdoid”, “NUT”, “SMARCB1/INI1/BAF47”.
- Criteria for SD-TST were
  - Undifferentiated round cell and/or rhabdoid morphology
  - SMARCA4 loss by IHC
  - Lack of epithelial adhesion molecule claudin-4
- 45 Conventional lung NSCCs (SD-NSCC) (comprising predominantly adenocarcinomas) with SMARCA4 truncating mutations and SMARCA4 loss by immunohistochemistry were selected from the retrospective search of cBioPortal.

IHC
- All tumors (SD-TST and SD-NSCC) were analyzed for expressions of
  - SMARCA4, SMARCA2, claudin-4, SALL4, and CD34.

Molecular and Cytogenetic Studies
- 16 SD-TSTs and 45 SD-NSCCs were analyzed by NGS platform for somatic mutations in cancer genes and for tumor mutation burden (TMB).
- FISH for SMARCA4(19p13) locus was performed.

Results:

Clinicoradiologic Characteristics
- SD-TST
  - Male predominant (73%); 21/22 Smokers, most heavy smokers; >50 years but also young (30-50 years)
  - Primary tumor type large: (2.2-18.3cm); Highly PET avid; 91% Stage IV disease
    - Sites of mets: lymph nodes, bone and adrenal.
Sarcomatoid areas were mostly round cells with variable amounts of rhabdoid tumors (17/22 specimens were core biopsies).

5/22 had conventional NSCC foci present.

IHC: All 22 had complete loss of SMARCA4 and 18/22 loss of SMARCA2;-positive: Synaptophysin 16/22; Patchy, diffuse-EMA; SALL4, CD34—20-30%; Negative: claudin 4; keratins, NUT and retained expression of SMARCB1; Ki67: mean 79%.

Features of SD-TST vs SD-NSCC

- SD-TST: > males; heavier smoking history; larger size (9cm vs 3 cm); much worse prognosis; do not met to the brain; have bulky peritoneal mets.; claudin 4-, most SMARCA2-deficient.
- SD-NSCC—claudin 4-positive, SMARCA2-proficient.
- Sharp loss of SMARCA2 in transition from carcinoma to sarcoma areas in SD-TST and foci of NSCC.

Genomic Signature

- 16 SD-TST analyzed, mostly by NGS.
- SD-TST had dominant smoking/tobacco signature (88%) vs 64% for SD-NSCC; control group of conventional sarcomas revealed no smoking signature (0/44).
- SMARCA4 gene alterations in 14/16; FISH for SMARCA4 revealed detectable alterations in 7/16 cases.
- TMB was comparable in both SD-TST and SD-NSCC.

Nosologic Relationship of Sarcoma vs Carcinoma

- 5/22 SD-TST have a NSCC component.
- Focal expression of NSCC markers (TTF-1 or p40).
- Smoking history with emphysema in all 21/22 SD-TST.
- Smoking related genomic alterations (dominant smoking signature; high TMB).
- Pattern of metastatic disease is more in line with carcinoma than sarcoma.

Take Home Messages: A tour de force by Dr. Rekhtman and team. The study reveals an abundance of clinicopathologic and genomic evidence that supports the theory that thoracic SMARCA4-deficient sarcomatoid tumors are primarily smoking-related, undifferentiated/de-differentiated carcinomas rather than primary thoracic sarcoma. Also, SD tumors in the thorax (SDS-TST and SD-NSCC) can be distinguished from each other by morphology (sarcoma element in the former) and IHC: SD-TST: claudin 4 neg and SMARCA2 deficient; SD-NSCC: claudin 4+ and SMARCA2 proficient. Also, despite the wide spread involvement of the mediastinum, the authors propose that most SD-TST arise within the lungs. Studies are in progress for optimal clinical management.
Use of Programmed Death ligand-1 (PD-L1) Staining to Separate Sarcomatoid Malignant Mesotheliomas from Benign Mesothelial Reactions.
Arch Pathol Lab Med 2020; 144:185-188.

**Background:** Separating reactive from malignant mesothelial proliferations is important, but can be difficult. Immune checkpoint inhibitors (CPIs) block T cells and macrophages from attacking normal cells as foreign cells. In normal cells, other than inflammatory cells, the expression of these CPIs is below the limits of detection by antibodies. Tumor cells can also express CPIs to block these attacks, but express these antigens at levels that are detectable by antibodies.

**Purpose:** Investigate the use of immunostaining for the checkpoint inhibitor PD-L1 as a method of separating benign from malignant mesothelial proliferation.

**Study Design:**
- Retrospective study of from University of British Columbia and Vancouver Coastal Health archives.

**Methods:** Tissue microarrays of reactive mesothelial proliferations (RMP), epithelioid (EMM) and sarcomatoid (SMM) mesotheliomas

**Tissue Samples—Microarrays:**
- 20 RMP-E (epithelial type) samples
- 20 RMP-S (spindle cell type) samples
- 27 EMM samples
- 6 SMM samples

**Tissue Samples—Whole Sections**
- 10 RMP-S
- 10 SMM

**Immunohistochemistry and Scoring**
- PD-L1 (22C3, Dako) with antigen retrieval
- Membrane staining only
  - Allred and H-score 12-point system
    - Cell proportion score: (0-4) x Staining intensity: (0-3) = 0-12
    - 0=negative; 1-2=weak; 3-8=moderate; 9-12=strong

**Results:**

**PD-L1 Staining Scores**

**Microarrays**
- RMPs: Both epithelial and spindle cell were mostly negative or rare weakly positive
- EMM: No statistical difference in staining with RMP-E
- SMM: Highly statistically significant difference with RMP-S

**Whole Sections**
- RMP-S: negative=8; weak=2
• SMM: negative=1; weak=0; moderate=2; strong=7

**Take Home Messages:** PD-L1 (22C3 clone) may be a helpful discriminator between reactive spindle cell mesothelial proliferations (i.e. organizing pleuritis) and sarcomatoid mesothelioma. It is probably not useful in distinguishing EMMs from reactive epithelioid mesothelial proliferations. The study is somewhat small and needs higher numbers and continued fine-tuning of the scoring system----in the spindle cell proliferations, it appears that strong staining is diagnostic of a SMM, but weak staining pattern in the setting of RMM-S versus SMM is not helpful, i.e. it does not rule out a mesothelioma.

**Usefulness of Methylthioadenosine Phosphorylase and BRCA-Associated Protein 1 Immunohistochemistry in the Diagnosis of Malignant Mesothelioma in Effusion Cytology Specimens.**

**Background:** The separation of benign and malignant mesothelial proliferations is difficult on effusion cytology. The gene for methylthioadenosine phosphorylase (MTAP) is located close to the CDKN2A gene and has been shown to be frequently deleted in tandem with CDKN2A in patients with malignant mesothelioma. Immunohistochemistry for MTAP is an established marker of malignancy in mesothelial proliferations in biopsies, but its use in cytology specimens is unknown.

**Purpose:** This study examined the use of immunohistochemistry for MTAP in differentiating reactive mesothelial proliferations from malignant mesotheliomas in effusion cytology specimens. The loss of nuclear BRCA-associated protein 1 (BAP1), which is very specific but only moderately sensitive for a diagnosis of mesothelioma, was also evaluated for comparison.

**Methods:**
**Study Design:** Retrospective study of cohort of available mesothelioma cases with matched positive effusion cytology specimens in the University of British Columbia and the University of Pittsburgh tissue archives from 2014-2018.

**Cases and Cell Block Preparation**
• 24 cases: Malignant pleural effusion cytology specimens with cell block
  o 14 cases with corresponding surgical biopsy proving mesothelioma
  o 10 cases with only cytologic diagnosis and also clinical/radiologic data completely consistent with malignant mesothelioma
• 15 cases: Control group of reactive mesothelial proliferations
• Unfixed pleural fluid centrifuged
  o ThinPrep slide
  o Cell block

**Immunohistochemistry**
• MTAP (clone 2G4; Abnova Corp)
  o Scoring
    ▪ Cytoplasmic: intact with >25% cells staining; lost: 0-24% staining
    ▪ Nuclear: Scored: Intact (100%); partial, or lost (0% staining)
• BAP1 (clone C4; Santa Cruz)
  o Nuclear staining: Intact (100%); partial, or lost (0% staining)

**Fluorescence in Situ Hybridization**
• CDKN2A: chromosome 9 centromeric probe
• At least 60 cells were scored
• Average # copies/cell
• Case was positive if homozygous deletion was identified in at least 20% of nuclei.

**Results:**

**MTAP**

*Cytoplasmic staining*
• Three (3) cases excluded due to insufficient tissue or lack of internal control; total of 21 cases were used for the analysis.
• 7/21 demonstrated complete loss of MTAP by IHC.
• 11/21 demonstrated no loss of cytoplasmic staining;
• 3 had partial loss
  o Same pattern seen in matching surgical biopsy
• 15/15 reactive mesothelial cytology specimens: No loss of MTAP by IHC
• Overall: Malignant Mesothelioma by cytoplasmic staining:
  o Sensitivity: 33%; Specificity: 100%.
• Statistically significant findings at P=.03.

*Nuclear staining*
• No statistical significance for complete nuclear loss between reactive and malignant cytology specimens.

**BAP1**
• 13/19 had loss of nuclear staining consistent with mesothelioma
  o Sensitivity: 68%; Specificity: 100%

**MTAP/BAP1 by IHC pm cytology specimen**
• 15/21 malignant mesothelial cytology specimens were positive for either one or the other.
• MTAP diagnosed 1/15 mesotheliomas that BAP1 did not diagnose on cytology

**MTAP with CDKN2A FISH**
• 5/7 cases with paired surgical specimen had positive MTAP IHC when CDKN21 FISH confirmed mesothelioma

**BAP1/ with CDKN2A FISH**
• 14/14 cases with paired surgical specimen had BAP1 positivity CDKN21 FISH confirmed mesothelioma
Take Home Messages: MTAP (33%) is not as sensitive of a marker as BAP1 (68%) in distinguishing reactive versus malignant mesothelial proliferations in cytology effusion specimens. Their combined sensitivity in these specimens is (71%). Specificity of each is 100%. Given this limited sensitivity, a failure to detect loss of either MTAP or BAP1 does not make the process benign and further workup is needed.

Articles for Notation

Neoplastic

Effects of Decalcifying Agents of Variable Duration on PD-L1 Immunohistochemistry.
Summary: The effects of four decalcifying specimens on PD-L1 (22C3 clone) expression in 10 placenta specimens (strong expressers) and 10 lung specimens (lower expressers) was measured. Two gentler solutions (EDTA and FA/MC) and two stronger HCL-based solutions were used as decalcification solutions. Using microarray technology and with a comparison to no decalcification, they measured % positive cells and change in intensity of staining. The strength and duration of treatment directly affect PD-L1 expressions with the gentlest solution (EDTA) and shortest duration causing minimal to no change compared to the no decalcification. The findings were found in both placenta and lung.
Take home message: Decalcification diminishes PD-L1 expression, as we all expected. The longer the decal, the less expression. EDTA is the gentlest. Interestingly, they did not do this using neoplastic tissue.

Highly accurate DNA-based detection and treatment results of MET exon 14 Skipping Mutations in Lung Cancer.
Summary: Non-squamous NSCLC specimens were examined for the presence of MET exon 14 skipping mutation (METex14del), which is known to drive non-small cell lung cancer in 1.3%-5.7% of NSCLC. This is important because cMET inhibitors may be effective in these tumors. They have evaluated tumors using a DNA-based analysis instead of the standard RNA-based analysis, which can be problematic due to acquiring sufficient RNA for this assay. The DNA assay is commercially available, but detects only 63% of known METex14del. The authors report that an in silico analysis detected 96% of reported METex14 alterations in a cohort of in 46 patients with proven METex14 del by NGS. Five (5) of the 46 patients with this mutation were treated with crizotinib. Four (4) of these treated patients achieved disease control, (3 with partial responses and 1 with stable disease), supporting the need to look for this mutation in NSCLC patients.
Take home message: A small trial, but encouraging results; a larger trial is needed to know if this therapeutic response is real. Interesting also that resistance mutations arose in two of the 5 treated patients.
**Concomitant genomic alterations in KRAS mutant advanced lung adenocarcinoma.**

**Summary:** KRAS mutations as a driver mutation in NSCLC is common and has varied clinical presentations. This study explored the potential clinical impact of coexisting alterations in patients with KRAS mutations. Samples from a cohort of 69 lung adenocarcinoma patients treated with platinum doublet as first-line therapy were assessed for KRAS mutations. 37 of these patients had advanced KRAS mutant and 32 had KRAS wild type 3. TP53 were more frequent in KRASwt than in KRASm as were STK11 mutations. FGFR3 were found only with KRASm. None of these had an effect on survival. Further evaluation of the effect of these co-alterations may be helpful for more accurate patient stratification for therapy.

**Take home message:** KRAS mutations are prevalent, yet no effective therapies are currently present. A strategy to further stratify these mutations may help find more specific therapies for at least subsets of these patients.

**Mucinous Lung Adenocarcinoma, Particularly Referring to EGFR-mutated Mucinous Adenocarcinoma.**
Wakejima R et al, Pathology International 2020;70:72-83.

**Summary:** This study evaluated mucinous adenocarcinomas that are part of mucinous adenocarcinomas of common types and invasive mucinous adenocarcinoma and colloid adenocarcinoma with nonmucinous adenocarcinomas. Of 1159 invasive adenocarcinomas, they found 189 mucinous adenocarcinomas and 970 nonmucinous adenocarcinomas. 20% of the mucinous adenocarcinomas were EGFR-mutated and 54% of the nonmucinous adenocarcinomas were EGFR-mutated. In comparing the EGFR-mutated mucinous adenocarcinomas (189) to the EGFR-mutated nonmucinous adenocarcinomas (100), they found that the mucinous EGFR-mutated adenocarcinomas had no female predominance, lower grades of histology, lower TTF-1 and higher HNF-4a expressions when compared to the nonmucinous cohort. Also, mucin production was an independent prognostic factor in this study. This may suggest a different tumorigenic pathway for these tumors.

**Take home message:** Mucinous adenocarcinomas may be different biologically than non-mucinous adenocarcinoma when looking only at those with EGFR mutations. The criteria for mucinous versus nonmucinous is somewhat unclear and that criteria may need tightening up if this question is further explored.

**Non-neoplastic**

**Poor Outcomes in Carriers of the RNF213 variant (p.Arg4810Lys) with pulmonary arterial hypertension.**

**Summary:** Whole-exome sequencing and direct sequencing were used to examine the genomes of 11 patients with idiopathic PAH. The RNF213 p.Arg4810Lys allele is known to confer a risk of extracranial vascular diseases, including peripheral pulmonary stenosis. This study examined a
cohort of 11 patients with this genetic variant. They were found to have poor clinical outcomes, suggesting lung transplantation early in the disease may be required. **Take home message:** The genetic mutations association with PAH are expanding beyond BMPR2, helping to clarify both the diagnosis and, in some cases, the prognosis in these patients.

**Ultrastructural Changes in Pulmonary Allografts with Antibody-Mediated Rejection.**
**Summary:** Antibody-mediated rejection (AMR) in lung transplants remains a significant cause for allograft loss. Given the nonspecific pathologic findings, a definitive and timely diagnosis is challenging. This study takes cues from the renal transplant literature and evaluates the ultrastructural changes in pulmonary allografts with AMR with the hope of finding more specific diagnostic features. Biopsies from 12 patients with AMR were evaluated and compared with both acute cellular rejection (ACR) biopsies and non-transplant controls. When compared to ACR controls, AMR biopsies revealed increased endothelial swelling, vacuolization and neutrophil margination. A total electron microscopy (EM) score was developed and was significantly higher in AMR versus ACR biopsies. The EM score did not correlate with C4d expression.

**Take home message:** EM can help with more specific diagnoses for AMR in lung transplant biopsies. A larger cohort is needed to validate. Most importantly, this could help with retrospective studies where archival tissue from clinically-validated AMR patients can be used to further define the specific morphologic features of AMR.

**Performance of Finnish biobanks in Nationwide Pulmonary Carcinoid Tumour Research.**
**Summary:** Finland has a hospital-integrated biobank system that administers millions of formalin-fixed paraffin-embedded tissue samples collect for clinical diagnostics. The study examined 224 tumor samples with appropriate patient data. This represented 88% of the patient cases registered at the Finnish Cancer Registry. The study identified 6 prognostic factors for shorter survival: age over 56, tumor size over 2.5cm, atypical histology, Ki-67 >2.5%, hilar/mediastinal LN involvement and metastatic disease.

**Take home message:** A national biobank infrastructure provided material for the study of pulmonary carcinoids in Finland. Expansion of additional services that would allow for more sophisticated analyses (i.e. molecular analyses) should be considered, but a wonderful asset for Finnish pathologists!

**Case Reports and Letters**

**Vaping-related Lung Injury in an Adolescent.**
Lu MA et al, Am J Respir Crit Care Med 2020;201:481-482.

**Take home message:** Acute fibrinous organizing pneumonia (AFOP) is added to the list of acute lung injury pathology documented in this setting.

**Take home message:** A good teaching case that reminds clinicians that all joint pain is not rheumatologic in nature.


**Take home message:** A nicely illustrated case of GLILD-CVID in various organs, including the eye, despite treatment-controlled pulmonary GLILD.

Programmed death ligand 1 immunohistochemistry: can we agree on this? Sholl LM. Histopathology 2020;76:189-190.

**Take home message:** A clearly written summary of where we are with PD-L1 reporting in a variety of tumors.