<u>**Pulmonary Pathology Journal Club**</u>
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<u>**Articles for Discussion**</u>
1. Cecchini MJ, Tarmey T, Ferreira A, at al. **Pathology, Radiology, and Genetics of Interstitial Lung Disease in Patients With Shortened Telomeres.** Am J Surg Pathol. 2021 Jul 1;45(7):871-884.

Background: ILDs in patients with shortened telomeres have not been well characterized. The authors describe demographic, radiologic, histopathologic, and molecular features, and p16 expression in patients with telomeres ≤10th percentile (shortened telomeres) and compare them to patients with telomere length >10th percentile.

Methods: Lung explants, wedge biopsies, and autopsy specimens of patients with telomere testing were reviewed independently by 3 pathologists using defined parameters. High-resolution computed tomography scans were reviewed by 3 radiologists. p16-positive fibroblast foci were quantified. A multidisciplinary diagnosis was recorded.

Results: Patients with shortened telomeres (N=26) were morphologically diagnosed as UIP (N=11, 42.3%), chronic HP (N=6, 23.1%), PPFE, fibrotic NSIP, DIP (N=1, 3.8%, each), and fibrotic ILD not otherwise specified (N=6, 23.1%). Patients with telomeres >10th percentile (N=18) showed morphologic features of UIP (N=9, 50%), chronic HP (N=3, 16.7%), fibrotic NSIP (N=2, 11.1%), or fibrotic ILD, not otherwise specified (N=4, 22.2%). Patients with shortened telomeres had more p16-positive foci (P=0.04). The number of p16-positive foci correlated with outcome (P=0.0067). Thirty-nine percent of patients with shortened telomeres harbored telomere-related gene variants. Among 17 patients with shortened telomeres and high-resolution computed tomography features consistent with or probable UIP, 8 (47.1%) patients showed morphologic features compatible with UIP; multidisciplinary diagnosis most commonly was IPF (N=7, 41.2%) and familial pulmonary fibrosis (N=5, 29%) in these patients.

Conclusion: Patients with shortened telomeres have a spectrum of fibrotic ILDs. They often demonstrate atypical and discordant features on pathology and radiology leading to diagnostic challenges.

Take home message: As with patients having familial forms of ILD, patients with shortened telomeres can develop a broad spectrum of fibrotic ILDs that can have atypical and discordant radiologic and pathologic features.

2. Lee JS, La J, Aziz S, et al. **Molecular markers of telomere dysfunction and senescence are common findings in the usual interstitial pneumonia pattern of lung fibrosis.** Histopathology. 2021 Jul;79(1):67-76.

Background: IPF is a genetically mediated, age-associated, progressive form of pulmonary fibrosis characterized pathologically by a UIP pattern of fibrosis. The UIP pattern is also found in pulmonary fibrosis attributable to clinical diagnoses other than IPF, whose clinical course is similarly poor, suggesting common molecular drivers. This study investigates whether IPF and non-IPF UIP lungs similarly express markers of telomere dysfunction and senescence.

Methods: To test whether patients with IPF and non-IPF UIP share molecular drivers, lung tissues from 169 IPF patients and 57 non-IPF UIP patients were histopathologically and molecularly compared.

Results: Histopathological changes in both IPF and non-IPF UIP patients included temporal heterogeneity, microscopic honeycombing, fibroblast foci, and dense collagen fibrosis. Non-IPF UIP lungs were more likely to have lymphocytic infiltration, non-caseating granulomas, airway-centered inflammation, or small airways disease. Telomeres were shorter in alveolar type II (AECII) cells of both IPF and non-IPF UIP lungs than in those of age-similar, unused donor, controls. Levels of molecular markers of senescence (p16 and p21) were elevated in lysates of IPF and non-IPF UIP lungs. Immunostaining localized expression of these proteins to AECII cells. The mucin 5B (MUC5B) gene promoter variant minor allele frequency was similar between IPF and non-IPF UIP patients, and MUC5B expression was similar in IPF and non-IPF UIP lungs.

Conclusion: Molecular markers of telomere dysfunction and senescence are pathologically expressed in both IPF and non-IPF UIP lungs. These findings suggest that common molecular drivers may contribute to the pathogenesis of UIP-associated pulmonary fibrosis, regardless of the clinical diagnosis.

Take home message: Not surprisingly, lungs from patients with IPF and non-IPF UIP show some histologic differences. However, the presence of shortened telomeres, molecular markers of senescence, and MUC5B promoter variants and MUC5B expression were similar in both groups, suggesting common drivers of disease when patients have UIP regardless of the clinical diagnosis.

3. Roden AC, Vrana JA, Koepplin JW, et al. **Comparison of In Situ Hybridization, Immunohistochemistry, and Reverse Transcription-Droplet Digital Polymerase Chain Reaction for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Testing in Tissue.** Arch Pathol Lab Med. 2021 Jul 1;145(7):785-796.

Background: Small case series have evaluated SARS-CoV-2 detection in FFPE tissue using rtPCR, IHC, and/or RNA ISH. The authors sought to compare droplet digital PCR, IHC, and RNA ISH to detect SARS-CoV-2 in FFPE tissue in a large series of lung specimens from COVID-19 patients.

Methods: Droplet digital PCR and RNA ISH used commercially available probes; IHC used clone 1A9. Twenty-six autopsies of COVID-19 patients with FFPE tissue blocks of 62 lung specimens, 22 heart specimens, 2 brain specimens, and 1 liver, and 1 umbilical cord were included. Control cases included 9 autopsy lungs from patients with other infections/inflammation and virus-infected tissue or cell lines.

Results: Droplet digital PCR had the highest sensitivity for SARS-CoV-2 (96%) when compared with IHC (31%) and RNA ISH (36%). All 3 tests had a specificity of 100%. Agreement between droplet digital PCR and IHC or ISH was fair ($\kappa = 0.23$ and $\kappa = 0.35$, respectively). Agreement

between IHC and ISH was substantial (κ = 0.75). Interobserver reliability was almost perfect for IHC (κ = 0.91) and fair to moderate for ISH (κ = 0.38-0.59). Lung tissues from patients who died earlier after onset of symptoms revealed higher copy numbers by droplet digital PCR (P = .03, Pearson correlation = -0.65) and were more likely to be positive by ISH (P = .02) than lungs from patients who died later. The authors identified SARS-CoV-2 in hyaline membranes, in pneumocytes, and rarely in respiratory epithelium. Droplet digital PCR showed low copy numbers in 7 autopsy hearts from ProteoGenex Inc. All other extrapulmonary tissues were negative.

Conclusion: Droplet digital PCR was the most sensitive and highly specific test to identify SARS-CoV-2 in lung specimens from COVID-19 patients.

Take home message: Droplet digital PCR is the best method for detecting SARS-CoV-2 in FFPE tissue, because it is highly sensitive and highly specific. IHC and RNA ISH are both highly specific, but their sensitivity is much lower.

4. Kinoshita Y, Hamasaki M, Matsumoto S, Yoshimura M, Sato A, Tsujimura T, Kamei T, Kawahara K, Iwasaki A, Nabeshima K. **Fluorescence in situ hybridization detection of chromosome 22 monosomy in pleural effusion cytology for the diagnosis of mesothelioma.** Cancer Cytopathol. 2021 Jul;129(7):526-536.

Background: Pleural mesothelioma is characterized by mutations in several genes, including CDKN2A/p16, BAP1, and NF2 in the 22q12 locus. Recent studies indicate that FISH detects hemizygous loss of NF2 in tissue specimens of pleural mesothelioma. The authors investigated whether NF2 FISH, either alone or in combination with other diagnostic assays (CDKN2A/p16 FISH, MTAP IHC, and BAP1 IHC), effectively distinguishes mesothelioma cells from reactive mesothelial cells in cell blocks prepared from pleural effusions.

Methods: FISH assays were used to examine the deletion status of NF2 and CDKN2A/p16, and IHC was used to determine the expression of MTAP and BAP1 in cell blocks from 54 cases with mesothelioma and 18 cases with reactive mesothelial cells.

Results: Hemizygous NF2 loss (chromosome 22 monosomy or hemizygous deletion) showed 51.9% sensitivity (48.1% for chromosome 22 monosomy and 3.7% for hemizygous deletion) and 100% specificity in differentiating mesothelioma cells from reactive mesothelial cells. Combinations of NF2 FISH, CDKN2A/p16 FISH, and BAP1 IHC assays yielded greater sensitivity (98.1%) than any assay alone (CDKN2A/p16 FISH, 61.1%; MTAP IHC, 52.8%; or BAP1 IHC, 60.4%). The level of hemizygous NF2 loss in cell blocks positively correlated with that in corresponding tissues. Furthermore, to overcome cytologic specimen-specific challenges, FISH combined with cytokeratin AE1/AE3 immunofluorescence was necessary in 25.9% of mesothelioma cases for FISH assessment of predominantly scattered mesothelioma cells.

Conclusion: NF2 FISH alone or in combination with other diagnostic assays effectively differentiates mesothelioma cells from reactive mesothelial cells in cell blocks prepared from pleural effusions.

Take home message: NF2 FISH may be worth adding to your list of useful studies to distinguish malignant from reactive mesothelial proliferations.

Articles for Notation

Neoplastic

1. Eccher A, Girolami I, Lucenteforte E, et al. Diagnostic mesothelioma biomarkers in effusion cytology. Cancer Cytopathol. 2021 Jul;129(7):506-516.
Summary: This is a nice review paper, highlighting biomarkers that can be used in effusion specimens to distinguish mesothelioma from reactive mesothelial proliferations, and discussing the limitations of these biomarkers. Biomarkers discussed include GLUT1, IMP3, BAP1, MTAP, and CDKN2A/p16.
Take home message: Nothing new here. Just a nice review for those who sign out pleural fluid specimens.

2. Agaimy A, Daum O, Michal M, et al. Undifferentiated large cell/rhabdoid carcinoma presenting in the intestines of patients with concurrent or recent non-small cell lung cancer (NSCLC): clinicopathologic and molecular analysis of 14 cases indicates an unusual pattern of dedifferentiated metastases. Virchows Arch. 2021 Jul;479(1):157-167.
Summary: The authors present 14 cases of metastatic undifferentiated large cell/rhabdoid carcinoma presenting in the bowel of patients with concurrent or recent NSCLC. Some but not all cases had a similar undifferentiated component in the lung biopsy, and half of the cases had loss of at least one SWI/SNF subunit. SMARCA2 loss was most frequent and was combined with SMARCA4 loss in one case. PBRM1 loss was observed in one tumor.
Take home message: Dedifferentiated NSCLC with loss of SMARCA4 or other SWI/SNF complex members can metastasize to strange places, such as the GI tract.

3. Yang SR, Chang JC, Leduc C, et al. Invasive Mucinous Adenocarcinomas With Spatially Separate Lung Lesions: Analysis of Clonal Relationship by Comparative Molecular Profiling. J Thorac Oncol. 2021 Jul;16(7):1188-1199.
Summary: To establish the clonal relationship (if any) between spatially separate lesions of invasive mucinous adenocarcinoma, genomic analysis with next-generation sequencing was performed on 2 separate lesions in 24 patients, including 19 with contralateral lesions. Driver mutations were shared in all but one case.
Take home message: Nearly all invasive mucinous adenocarcinomas with multifocal lesions represent a single neoplasm with intrapulmonary spread.

4. Zens P, Bello C, Scherz A, et al. A prognostic score for non-small cell lung cancer resected after neoadjuvant therapy in comparison with the tumor-node-metastases classification and major pathological response. Mod Pathol. 2021 Jul;34(7):1333-1344.
Summary: The prognostic accuracy of the TNM classification for NSCLC after neoadjuvant therapy is poorly understood, and the cut-off percentage at which tumor regression or major pathological response predicts overall survival and disease-free survival has not been established. This study aimed to validate a novel combined prognostic score and establish the optimal cut-off for major pathologic response after therapy.
Take home message: Pathologic tumor response after neoadjuvant therapy for NSCLC is a relevant prognostic factor, in addition to the TNM classification. The optimal cut-off for major pathologic response for lung adenocarcinoma was 65%, and for lung non-adenocarcinomas was 10%.

Non-neoplastic
1. Evert K, Dienemann T, Brochhausen C, et al. Autopsy findings after long-term treatment of COVID-19 patients with microbiological correlation. Virchows Arch. 2021 Jul;479(1):97-108.
Summary: In 17 fatal cases of COVID-19 where autopsies were performed, most cases showed DAD. Several cases also showed evidence of superimposed fungal pneumonia, and 60% of cases showed organizing thrombi.
Take home message: Nothing new here, and nothing surprising.

Case Reports and Letters
Neoplastic
1. Hyde R, Wynne J, Sriram P, et al. A 67-Year-Old Woman With Abdominal Pain, Paresthesia, and Rapidly Expanding Lung Nodule. Chest. 2021 Jul;160(1):e29-e34.
Summary: A good review on mucous gland adenoma, bronchial papilloma, pulmonary hamartoma, alveolar adenoma, bronchiolar adenoma, PECOMA, and sclerosing pneumocytoma.

2. Watabe Y, Ujiie H, Matsuno Y, et al. Rare Diagnosis of a Multilobular Pulmonary Mass. Chest 2021 Jul;160(1):e63-e67.
Summary: An interesting case of solitary pulmonary cavernous lymphangioma.

Non-neoplastic
1. Qin X, Chen X, Deng Y, et al. A 12-Year-Old Girl Presenting With Recurrent Dyspnea and Pulmonary Ground-Glass Opacities. Chest 2021 Jul;160(1):e45-50.
Summary: An interesting case of anti-Jo-1 anti-synthetase syndrome.

2. Chong WH, Saha B, Ibrahim A, et al. Dyspnea in a 57-Year-Old Man With Recent Viral Illness. Chest. 2021 Jul;160(1):e19-e24.
Summary: An interesting case of COP.

3. Manion M, Sereti I. A 34-Year-Old Man With HIV/AIDS and a Cavitary Pulmonary Lesion. Chest 2021 Jul;160(1):e35-e38.
Summary: An interesting case of Cryptococcus neoformans infection.

4. Kattih Z, Birnbaum B, Gabra N, et al. Intermittent Cough and Hemoptysis With Tree-in-Bud Opacities on Imaging. Chest. 2021 Jul;160(1):e69-e75.
Summary: An interesting case of idiopathic diffuse pulmonary ossification.

5. Perlepe G, Karetsi E, Papamichali R, et al. A 72-Year-Old Woman With Multiple Pulmonary Nodules and a History of Malignancy. Chest. 2021 Jul;160(1):e57-e61.
Summary: An interesting case of DIPNECH syndrome.

6. Saha BK, Bonnier A, Chong WH, et al. A 75-Year-Old Woman With Pulmonary Nodules and Dyspnea. Chest. 2021 Jul;160(1):e51-e56.

Summary: Another interesting case of DIPNECH syndrome.

7. Pasula S, Chandrasekar P. Spontaneous Hemoptysis in a Patient With COVID-19. Chest. 2021 Jul;160(1):e39-e44.
Summary: An interesting case of invasive pulmonary aspergillosis due to A. niger infection in an immunocompetent patient with COVID-19 pneumonia.

8. Goondi D, Franko A, Johannson KA. Pleuroparenchymal Fibroelastosis after Liver Transplantation. Am J Respir Crit Care Med. 2021 Jul;204(2):222-223.
Summary: The title says it all. A case of PPFE developing 3 years after liver transplantation.

9. Rubin J, Sclafani A, Helland TL, et al. Pulmonary Hemosiderosis with Calcification Associated with IgA Nephropathy. Am J Respir Crit Care Med. 2021 Jul;204(2):e24-e25.
Summary: An interesting case of pulmonary hemosiderosis and calcification after chronic recurrent DAD secondary to IgA nephropathy.

10. Ghalib S, Chopra A, Fantauzzi JP, et al. A Young Woman With Recurrent Episodes of Fever and Cough. Chest. 2021 Jul;160(1):e25-e28.
Summary: An interesting case of bronchopulmonary sequestration.