

PULMONARY PATHOLOGY JOURNAL CLUB
(June 2022 Articles)

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Discussion articles

Rose CS et al. Small airways and airspace inflammation and injury distinguish lung histopathology in deployed military personnel from healthy and diseased lungs. Hum Pathol 2022; 124:56-66.

Prepared and presented by Dr. William Perry (Thoracic Pathology Fellow 2022-2023)

Purpose: To characterize histopathologic findings in surgical lung biopsies from patients with persistent respiratory symptoms following military deployment and compare those findings to lung tissue from unused donor lungs (“normal controls”) and from patients with several lung diseases that affect small airways

Methods:

- Reviewed patients being evaluated for unexplained exertional dyspnea following military deployment between 2001-2017 to identify patients who underwent lung tissue sampling
- Identified 65 patients with lung tissue samples (63 surgical lung biopsies, 1 lobectomy, 1 cryobiopsy); collected basic demographic information, smoking history, and self-reported exposure frequency to sandstorms, smoke from burn pits, diesel exhaust particulates, and combat dust from mortar fire.
- Identified comparison tissue from unused donor lungs (n=10) and patients with chronic hypersensitivity pneumonitis (n=8), respiratory bronchiolitis (n=10), and post-transplant or autoimmune obliterative bronchiolitis (n=11)
- A pulmonary pathologist, blinded to the original diagnosis, used a scoring system to score a variety of histologic abnormalities in all cases including separate scoring of each different site (lobe) biopsied

Results:

- **Table 1: Demographics, smoking characteristics, and lung tissue sample types**
 - Deployers were overwhelming male (95%) and younger on average than all other groups (mean age 40.2 years)
 - 3% of deployers were current smokers and 40% were former smokers; comparison groups had lower percentage of current/former smokers or high amounts of missing smoking status data
 - Vast majority of cases had both upper and lobe specimens available for evaluation and many had middle lobe specimens as well
- **Table 2: Major histologic findings by group**
 - The most common findings in deployers were lymphocytic bronchiolar inflammation (90.8%), lymphocytic pleural inflammation (89.2%), smooth muscle hypertrophy (69.2%), and peribronchiolar metaplasia (67.7%)
 - Bronchiolar and/or interstitial granulomatous inflammation and moderate to severe emphysema were each seen in just under half of the deployer biopsies

- All other scored abnormalities were present in at least a subset of deployer biopsies although lymphoid follicles (in any compartment) and organizing pneumonia were both uncommon
- **Figure 6: Lobar distribution of major histologic findings in deployers**
 - Slight predominance of small airway injury in the lower lobes relative to upper lobes in deployer lung tissue

Take-home Message: Lung tissue from patients with unexplained exertional dyspnea following military deployment frequently shows lymphocytic bronchiolar and pleural inflammation, peribronchiolar metaplasia, and occasionally peribronchiolar granulomatous inflammation, indicating they often have significant histologic overlap with chronic hypersensitivity pneumonitis. In general, lung biopsies from this patient population displayed a wide variety of histologic abnormalities indicative of small airway and airspace injury but were non-specific when compared to other lung diseases affecting similar structures.

Bruehl FK et al. Does histological assessment accurately distinguish separate primary lung adenocarcinomas from intrapulmonary metastases? A study of paired resected lung nodules in 32 patients using a routine next-generation sequencing panel for driver mutations. J Clin Pathol 2022; 75:390-396.

Purpose: Assess the accuracy of histologic assessment to distinguish separate primary lung adenocarcinomas from intrapulmonary metastases, using the results of molecular testing as the determining factor for relatedness

Methods:

- Identified adenocarcinomas from patients with 2 resected lung nodules at Cleveland Clinic between 2008 and 2019
 - Exclusion criteria: Both nodules in the same lobe
- Slides from paired tumors independently reviewed by 3 pathologists, blinded to all data
 - Reported as “related” (intrapulmonary metastasis) or “unrelated” (separate primary lung adenocarcinomas) with analysis of patterns, nuclear grade, and presence/extent of mucinous features
 - If difference of interpretation on relatedness, majority opinion used for analysis
- Molecular testing performed on all tumors; determination of relatedness based upon:
 - Unrelated: Different driver mutations *or* 1 driver mutation and 1 wild-type (WT)
 - Related: Same *low frequency* driver mutation
 - Indeterminate: 1) Both WT; 2) Both have identical *high frequency* (HF) driver mutations; *or* 3) 1 driver mutation present, but other tumor not tested for that mutation in the setting of limited molecular testing

Results:

- 32 paired cases met inclusion criteria
- Table 1 details the results of histologic assessment
 - Disagreement in interpretation between pathologists in 6 (18%) cases
- Table 2 details driver mutation status and determination of molecular relatedness
 - 8 cases “indeterminate” for molecular relatedness; 4 HF mutations; 2 WT; 2 mutation not tested in 1 tumor
- Figure 2 reflects accuracy of histologic assessment in context of molecular findings
 - NO cases deemed unrelated by histology found to be related by molecular testing
 - 27% (4) of cases thought to be related on histology found to be *unrelated* by molecular assessment
 - All cases non-mucinous or mixed with same predominant pattern (table 3)
 - 2 cases correctly staged in original pathology report because pathologist aware of molecular test results prior to sign out
- No difference in overall survival between patients with intrapulmonary metastases and biologically unrelated tumors (figure 4)

Take-home message: If two tumors look different and you think that they are unrelated, than you are almost certainly correct. When tumors are morphologically similar in a patient with no other evidence of disease to suggest metastasis, you might consider performing molecular testing to sort out if the tumors are truly related to avoid over-staging/over-treating the patient.

Fuchs TL et al. A critical assessment of current grading schemes for diffuse pleural mesothelioma with a proposal for a novel mesothelioma weighted grading scheme (MWGS). Am J Surg Pathol 2022; 46:774-785.

Purpose: 1) Examine the reproducibility and prognostic value of the 4 main grading schemes for diffuse pleural mesothelioma (Table 1); and 2) propose a novel “Weighted Mesothelioma Grading Scheme” (MWGS), useful in risk stratifying patients regardless of histologic subtype or BAP1 status

Methods:

- Retrospectively reviewed all cases of diffuse pleural malignant mesothelioma in which the paraffin-embedded tissue block was adequate and available, diagnosed at Royal North Shore Hospital in Sydney between January 1990 and April 2021
 - Patient demographics and all causes of death recorded
 - Staging and treatment data not available
- For each case, 1 representative H&E-stained section independently reviewed by 3 pathologists, blinded to all data, who assessed 1) Histologic subtype (epithelioid, sarcomatoid, or biphasic); 2) Nuclear atypia – See Figure 1; 3) Mitotic count; and 4) Coagulative tumor necrosis – present or absent
- Immunohistochemistry for BAP1 performed on all cases

Results:

- 369 small biopsies (See Table 2 for clinical and pathologic characteristics)
 - 299 (81%) patients died during follow-up period
- Univariate analysis: Age > 74 years, non-epithelioid histology, retained BAP1 expression, mitotic count ≥ 5 per 2 mm², necrosis, and severe nuclear atypia significantly associated with worse overall survival
 - Multivariate analysis: All variables, except mitotic count significantly correlated with overall survival
- Kadota 2012 nuclear grading worked to stratify patients, but when subtype considered, only prognostically significant for sarcomatoid and biphasic types (Supp Fig 1A-D)
- Supplemental Figure 3 shows performance of 5-tiered system, which had no rank-ordered hierarchical relationship between advancing grade and overall survival
- Figure 2 details results of WHO grading system survival analysis
- M-N score stratified patients, but not applicable to all subtypes or when BAP1 retained (Supp Figure 2)
- Table 3 outlines proposed MWGS
 - Median survival = low: 17.1 mo.; intermediate: 10.1 mo., high: 4.1 mo. (Fig 3A)
 - Progressive increase in score correlated with worsening survival (Fig 4; Supp 5)
- Good interobserver agreement with assessment of nuclear grade being poor ($\kappa = 0.195$)

Take-home message: The MWGS achieves the author’s stated aim and contains no new histologic parameters that are not already being assessed in some combination in the existing grading systems, so it would be easy to implement, *but* it does require performing BAP1 on all cases.

Yang H et al. UHRF1 Immunohistochemical staining separates benign reactive spindle cell mesothelial proliferations from sarcomatoid mesotheliomas. Am J Surg Pathol 2022; 46:840-846.

Purpose: 1) Evaluate the use of ubiquitin-like with plant homeodomain and ring finger domains-1 (UHRF1) immunohistochemical stain in separating benign from malignant mesothelial proliferations; 2) investigate if there is a relationship between UHRF1 percent positivity and loss/retention of BAP1 and MTAP; and, 3) assess if it is helpful in differentiating sarcomatoid mesothelioma from sarcomatoid carcinoma

Methods:

- UHRF1-AE1/AE3 dual stain (Figure 1)
- Applied to tissue microarrays (TMA): 29 benign epithelial mesothelial proliferations, 11 benign spindle cell mesothelial proliferations, 39 epithelioid mesotheliomas, 22 sarcomatoid mesotheliomas
- Applied to whole sections: 19 organizing pleuritis, 21 sarcomatoid mesotheliomas, including 5 desmoplastic mesotheliomas
- Used TMA with 18 sarcomatoid carcinomas of the lung
- Counted nuclear staining in keratin-positive cells
- BAP1 and MTAP immunostains performed on TMAs

Results:

- Table 1 details TMA “screening” results for UHRF1
 - Although statistically significant, there was a lot of overlap between reactive epithelial proliferations and epithelioid mesothelioma (figure 2A), so determined to be not diagnostically useful in this setting
 - Broad range of staining for sarcomatoid mesotheliomas (2.5-95%; figure 2A)
 - Using cutoff of >10%, sensitivity and specificity for sarcomatoid mesothelioma 76 and 100%, respectively
- Table 2 details results of whole section evaluation for spindled mesothelial proliferations
 - Cutoff of 9.3%, 100% sensitive and 100% specific
- No relationship between UHRF1 staining fraction and loss/retention of BAP1 or MTAP for sarcomatoid mesotheliomas
- 17 of 18 (94%) of sarcomatoid carcinomas show high fraction of UHRF1 staining, so this marker is not helpful in the setting of mesothelioma vs. carcinoma differential

Take-home message: You might consider adding UHRF1 to your immunohistochemical lab to aid in differentiating benign from malignant spindled mesothelial proliferations.

Articles for notation

Neoplastic lung disease

Batra U et al. IHC versus FISH versus NGS to detect ALK gene rearrangement in NSCLC: all questions answered? J Clin Pathol 2022; 75:405-409.

Take-home message: In this study, 58 consecutive, ALK IHC (D5F3 antibody)-positive cases of pulmonary non-small cell carcinoma underwent FISH and NGS testing to assess concordance and sensitivity of these modalities. The concordance of positive results for IHC and FISH was 75.9% ($n = 44$), IHC and NGS was 84.5% ($n = 49$), and NGS and FISH was 65.5% ($n = 38$). The sensitivity of NGS (87.5%) is greater than FISH (75.6%); therefore, the authors conclude that IHC should remain the preferred screened tool followed by confirmatory NGS for variant characterization.

Hou L et al. Prognostic and predictive value of the newly proposed grading system of invasive pulmonary adenocarcinoma in Chinese patients: a retrospective multicohort study. Mod Pathol 2022; 35:749-756.

Take-home message: Using the International Association for the Study of Lung Cancer (IASLC) Pathology Committee's proposed grading system for invasive adenocarcinomas as well (grade 1), moderately (grade 2), and poorly (grade 3) differentiated based upon predominant pattern and percentage of high-grade patterns, the authors retrospectively reviewed and re-classified 926 stage I lung adenocarcinomas to evaluate the prognostic impact and utility of this system in predicting response to adjuvant chemotherapy (ACT). They found that grade 3 tumors had significantly worse recurrence free survival (RFS) and overall survival (OS) compared to grade 1 and grade 2, and demonstrated that pathologic grading was an independent prognostic factor for both RFS and OS. Of the patients treated with ACT, the new grading system indicated a survival benefit in patients previously graded as grade 2 (i.e. acinar or papillary predominant) but upgraded to grade 3 using the new IASLC system.

Jian D et al. NSCLC subtyping in conventional cytology: results of the international association for the study of lung cancer cytology working group survey to determine specific cytomorphologic criteria for adenocarcinoma and squamous cell carcinoma. J Thorac Oncol 2022; 17:793-805.

Take-home message: The aim of this study was to improve the subtyping of NSCC on non-cell block cytology specimens obtained from lung lesions using cytomorphology alone, using machine learning applied to 119 cases reviewed by 13 expert cytopathologists. Using cytomorphology, the experts accurately subclassified 53% of cases as adenocarcinoma or squamous cell carcinoma, and squamous cell carcinomas without keratinization were the most frequently misclassified. Machine learning was not found to reduce rates of NSCC-NOS without increasing misclassification.

Perez-Johnston R et al. CT-based radiogenomic analysis in clinical stage I lung adenocarcinoma with histopathologic features and oncologic outcomes. *Radiology* 2022; 303:664-672.

Take-home message: In this work, 219 cases of stage I adenocarcinoma were assessed by CT-based radiomics, using 102 features that resulted in separation of tumors into 4 clusters based upon similar CT features with clusters correlating with different histologic patterns, presence of lymphovascular invasion, and spread through airspaces. Pathologist do not fear - - This tool might be applied as a preoperative predictive model for identification of patients with high-risk of recurrence/metastasis. Radiologists are not taking our jobs...yet.

Ruffini E et al. The international association of the study of lung cancer thymic epithelial tumor staging project: unresolved issues to be addressed for the next ninth edition of the TNM classification of malignant tumors. *J Thorac Oncol* 2022; 838-851.

Take-home message: Here, the authors offered a midterm report of unresolved issues in the classification of thymic tumors that emerged during the 2017-2020 term of thymic domain subcommittee. The issues were described in the context of the current 8th edition of TNM classification and new literature to consider for the upcoming 9th edition (expected in 2024), and included 1) geographic differences in thymic tumors across continents, 2) if thymomas and thymic carcinoma should be staged the same way, 3) the potential role of tumor size in T stage, 4) involvement of adjacent structures, 5) nodal involvement, and 5) advanced disease.

Takamochi K et al. Clinical relevance of PD-L2 expression in surgically resected lung adenocarcinoma. *Lung Cancer* 2022; 168:50-58.

Take-home message: Nine hundred-eighty cases of lung adenocarcinoma were evaluated for programmed death ligand 2 (PD-L2) expression by immunohistochemistry and these results were compared with PD-L1. Expression pattern of PD-L1, PD-L2, and combined PD-L1/PD-L2 were assessed in the contexts of clinical, pathologic, and molecular characteristics, and survival. Perhaps most interesting of all the parameters analyzed, unlike PD-L1, PD-L2 does not show any prognostic significance.

Tancoš V et al. Expression of programmed death ligand-1 protein in pulmonary squamous cell carcinoma correlates with tumor necrosis but not with tumor differentiation. *J Clin Pathol* 2022; 75:373-378.

Take-home message: The authors investigated the relationship between PD-L1 expression by immunohistochemistry and the histologic characteristics of pulmonary squamous cell carcinoma in 133 patient samples. While PD-L1 expression did not correlate with degree of differentiation defined by presence or absence of keratinization, increased expression was significantly associated with the presence of tumor necrosis, regardless of T stage.

Non-neoplastic lung disease

Flaifel A et al. Pulmonary pathology of end-stage COVID-19 disease in explanted lungs and outcomes after lung transplantation. Am J Clin Pathol 2022; 908-926.

Take-home message: This study details the pretransplant clinical course, pathologic findings, and posttransplant outcomes of 3 patients, who developed chronic respiratory failure due to coronavirus disease 2019 (COVID-19) and underwent lung transplant 8 to 11 months following acute infection. As perhaps anticipated, the lung explants showed the proliferative and fibrotic phases of diffuse alveolar damage (DAD) with the fibrosis resembling nonspecific interstitial pneumonia.

Molyneaux PL et al. CYFRA 21-1 predicts progression in idiopathic pulmonary fibrosis: a prospective longitudinal analysis of the PROFILE cohort. Am J Respir Crit Care Med 2022; 205:1440-1448.

Take-home message: The aim of this study was to evaluate CYFRA 21-1, a soluble cleavage fragment of cytokeratin (CK) 19, as a prognostic biomarker in patients with idiopathic pulmonary fibrosis (IPF). Immunohistochemistry using an antibody directed against CK 19 was applied to 20 IPF patient surgical lung biopsies and showed increased expression in hyperplastic epithelial cells in areas of fibrosis. Serum levels of CYFRA 21-1 were serially measured in IPF patients ($n = 359$) and higher concentrations were shown to correlate with worse survival.

Case report

Kataoka M et al. Coronavirus hunted in human pneumocytes and alveolar macrophages: a case report. Histopathology 2022; 80:1130-1134.

Take-home message: Transmission electron microscopy was performed on autopsy material from a 64-year-old woman who died of COVID-19-associated myocarditis with lung findings of DAD. As the title implies, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in type II pneumocytes, alveolar macrophages, and macrophages phagocytosing infected pneumocytes. The images from Figure 1 are featured on the cover of this month's edition of *Histopathology* – Pretty cool!

Letter to the editor

Nakashima J et al. A case-series of six autopsy cases of COVID-19 including three cases of cytomegalovirus coinfection. Pathol Int 2022; 72:358-360.

Take-home message: The title pretty much sums it up... All 3 of the patients with both SARS-CoV-2 and CMV were immunosuppressed.