PULMONARY PATHOLOGY JOURNAL CLUB
(October 2023 Articles)

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Discussion articles

Matsunaga T et al. A problem with clinical T factor in the 8th TNM edition: Prognosis and EGFR mutation status of small sized lung cancers with difficulty to measure the diameter of solid component in part-solid tumor. Lung Cancer 2023; 184:107354.

Prepared and presented by Dr. Heather Chen-Yost (Thoracic Pathology Fellow 2023-2024)

Purpose: The 8th TNM for lung cancer recommends for part-solid tumors (tumors with invasive and lepidic growth) using the maximum consolidation size for the clinical T descriptor, which can be difficult to measure. They aim to clarify how part-solid tumors should be classified in terms of prognosis and biologic behavior.

2013 paper:

- Part-solid tumors are difficult to measure due to the scattered and discontinuous consolidation

Methods:

- Reviewed 590 patients with surgically resected cT1N0M0 stage 1A non-small cell lung cancers (NSCLC).
    - Resection dates: January 2009 to October 2012
    - Had clinical stage IA- Maximum tumor size ≤3cm without lymph node metastasis or distant metastasis
    - Excluded lungs with emphysema
- CT scans were performed to evaluate the tumor and solid component diameters
    - Evaluated: Maximum tumor dimension, maximum dimension of consolidation, and distribution of ground-glass opacity (GGO)
    - Classified tumor distribution based off radiologic types
        - Islands: Consolidation was discontinuous
        - Reticular: Consolidation is mesh-like in GGO
        - Dense: Density between GGO and consolidation
    - Patients followed up every 3-6 months after surgery for 5 years
        - CT chest and upper abdomen: every 6 months
        - CT/MRI of brain, bone scintigraphy, or PET-CT every year for first 2 years
- Re-classified tumors based off 8th edition
- Other clinicopathologic findings:
    - Surgical procedure
    - Pathologic stage from 7th edition
    - Recurrence day- Based off radiologic relapse
    - Overall survival (OS): Time from day of surgery to death from any cause OR last follow-up
    - Cancer-specific survival (CSS): Time from day or surgery to death from lung cancer OR last follow-up

    - EGFR-mutation status- Routinely evaluated (found in 521 of 590 tumors)
- Stats
    - Medians: Chi-squared and Fisher's exact tests
    - Cumulative survival: Kaplan-Meier model
    - Survival rate: Log-rank test and Cox proportional hazards model

Results:

- **Patient Characteristics**
    - Distribution of stages: Lung cancers with scattered or mixed consolidation (LCSMC, n=79), cTis (n=99), cT1mi (n=52), cT1a (n=68), cT1b (n=166), and cT1c (n=126)
    - 277 men, 313 women; age range: 24 to 89
- **LCSMC in relation to other tumors**
    - Incidence of p-stage IA was 91.1%
    - Median maximum tumor size on CT scan placed it between cT1b and cT1a
    - Ratio of lobectomy between cT1a and cT1b
    - Frequency of lymphatic invasion placed between cT1a and cT1b
    - Frequency of vascular invasion between cT1mi and cT1a
    - Most common histologic subtype were lepidic adenocarcinoma (n=49, 62%), acinar (n=14, 17%)
    - STAS not observed
- **Prognosis of LSCMS compared to others**
    - 5-year OS: 92.4% (between cTmis and cT1a)
    - 5-year CSS: 96.1% (between cT1a and cT1b)
    - Prognosis significantly better than in cT1b (p=0.002), but no significant difference was found between LSCMS and cT1a tumors (p=0.169)
- **EGFR status compared to others**
    - Incidence of EGFR in LCSMS was 54.8%
    - No significant difference between LCSMC tumors and cT1a for frequency (p=0.778)
    - Higher incidence in LCSMCs than in cT1b tumors (p=0.077)

Take-home Message: Part-solid tumors are best classified as cT1a tumors based off prognosis, biologic behavior (lymphatic and vascular invasion), and EGFR status

Zombori-Tóth N et al. Proposal of a grading system for squamous cell carcinoma of the lung – the prognostic importance of tumor budding, single cell invasion, and nuclear diameter. Virchows Arch 2023; 483:393-404.

Prepared and presented by Dr. Heather Chen-Yost (Thoracic Pathology Fellow 2023-2024)

Purpose: Propose a grading system for lung squamous cell carcinoma (LSCC), using tumor budding, nuclear diameter, and single cell invasion.

- Compare grading system to other proposed systems
  - Kadota et al: Grade based off tumor budding and nuclear diameter
  - Weichert et al: Grade based off tumor budding and nest size

Methods:

- Patients with LSCC who underwent resection between 2010-2016 with follow-up (end date: July 1st, 2022)
- Clinical parameters recorded
  - Age, gender
  - Smoking habits
  - Type of surgery
  - Adjuvant therapy
  - Follow-up: OS, recurrence free survival (RFS)
- H&E-stained sections reviewed by three authors, blinded to clinical outcome. Pathologists recorded
  - Tumor size (mm)
  - Distance to resection margin (mm)
  - Spread through airspaces (STATS)
  - Number of mitosis/10 HPFs
    - Low: <15 mitoses/10HPFs
    - High: ≥15 mitosis/10HPFs
  - Nature of invasive front: Expansive vs infiltrative
  - Invasion: Vascular, lymphovascular, pleural
- Proposed three tier grading scheme:
  - Cumulative score between extent of tumor budding + presence of single cell invasion + nuclear diameter.
- Parameters in grading scheme
  - Tumor budding: Presence of isolated small tumor nests composed of less than 5 tumor cells are the invasive tumor front, surrounded by desmoplastic stroma
    - Total number of beds on 10 MPFs
    - Maximum number of buds in one hotspot MPF
    - Low: 0 buds/10 MPFs
      Medium: 1-14/10 MPFs
      High: ≥15 buds/10 MPFs
  - Minimal cell nest size: Smallest tumor cluster within tumor or invasive front, subclassified according to cell number
    - Single cell invasion

        - Small nest (2-4 tumor cells)
        - Intermediate nests (5-14 tumor cells0
        - Large nest (≥15 tumor cells)
    - Nuclear diameter: Compared to lymphocytes
        - Small (≤4 lymphocytes) vs Large (>4 lymphocytes)
- Stats:
    - Association between variables: Chi-square and Kruskal-Wallis tests
    - Impact morphologic variables had on OS and RS: Univariate Cox proportional hazards model. If significant-> Multivariate Cox proportional hazards model

Results:

- 220 total LSCC patients in study, median follow-up of 81 months
- Median age: 63.8 years. Median RFS: 19.3 months, OS: 23 months
- Parameters in grading scheme investigated associated with adverse prognosis
- Other associations with parameters

  Tumor budding
    - Associated with smoking history (p=0.003) and higher stage (p=0.031)
    - More often in keratinizing histologic subtype (58%)
    - Associated with infiltrative tumor border, smaller minimal cell nest size, single cell invasion, larger nuclear diameter, and invasion.

  Single cell invasion
    - More frequent in patients with higher nodal status and higher stage (p <0.001 both)
    - Associated with infiltrative tumor border, smaller minimal cell nest size categories, lymphovascular invasion.

  Large nuclear diameter
    - More frequent in smaller minimal cell nest size categories
- STATs associated with poor prognosis independent, but rare event
- Proposed grading system had highest AUC value in ROC curve analysis
    - Compared against Weichert and Kadota grading systems was better able to separate three prognostic categories

Take-home message: A three-tier grading scheme for LSCC based off tumor budding, presence of single-cell invasion and nuclear diameter correlates with prognosis

Bremmer F et al. Proteomic analysis identifies argininosuccinate synthase 1 and special AT-rich sequence binding protein 1 as reliable markers for the immunohistochemical distinction between WHO types A and B3 thymomas. Histopathology 2023; 83:607-616.

Purpose: Identify and validate markers that might help in differentiating type A and B3 thymomas

Methods:

- Candidate protein identification and validation performed by gel electrophoresis followed by mass spectrometry
    - Screening set: Snap-frozen samples of type A thymoma (*n* = 3) and type B3 thymoma (*n* = 3)
        - For all thymomas used in this study, WHO classification was based upon H&E diagnosis
- Antibodies for select candidate proteins applied to pilot set of *n* = 10 type A thymoma, *n* = 10 type B3 thymoma
- Validation set blocks immunostained with antibodies directed against ASS1 and SATB1
    - Validation set: 88 formalin-fixed, paraffin-embedded samples (*n* = 34 type A thymoma, *n* = 20 type B3 thymoma, *n* = 14 type AB thymoma, *n* = 4 type B1 thymoma, *n* = 10 type B2 thymoma, *n* = 6 thymic carcinoma)
- Immunofluorescence dual stains performed to confirm stained cell types as epithelial

Results:

- Mass spectrometry identified 49 proteins differentially expressed in type A vs. type B3 thymoma
    - From these, 8 potentially useful markers (listed on page 610) selected and antibodies applied to the pilot set
    - AT-rich sequence binding protein 1 (SATB1) and argininosuccinate synthase 1 (ASS1) selected for application to validation set
- Table 1 details staining pattern for all tumors tested

	Type A thymoma	Type B3 thymoma
ASS1 (cytoplasmic)	34 (100%) negative	20 (100%) positive
SATB1 (nuclear)	31 of 34 (92%) positive	19 of 20 (95%) negative

- Sensitivity/Specificity of ASS1 = 100%
- For SATB1, sensitivity is 91% and specificity is 95%
- In combination, these markers show 94% sensitivity and 98% specificity in discriminating type A and B3 thymomas
    - For the cases that did not stain as expected, there were no other findings to suggest incorrect classification

Take-home message: You might consider applying ASS1 and SATB1 immunohistochemical stains when trying to differentiate between type A thymoma and type B3 thymoma with spindle morphology, particularly on small biopsies.

Dacic S et al. International association for the study of lung cancer study of reproducibility in assessment of pathologic response in resected lung cancers after neoadjuvant therapy. J Thorac Oncol 2023; 18:1290-1302.

Purpose: Assess the reproducibility of the IASLC histologic criteria for pathologic response in resected NSCLC treated with neoadjuvant immunotherapy +/- chemotherapy among thoracic pathologists around the globe

Methods:

- Tumor bed slides from 6 clinical trials amassed, all representing NSCLCs resected from patients who received anti-PD-L1 therapy, anti-CLTA-4 therapy, or anti-PD-L1 therapy + chemotherapy
    - Exclusion criteria: Synchronous multiple tumor nodules
- H&E slides were digitized and reviewed by 2 groups of pathologists (11 pathologists total) blinded to all data and who underwent 3 online training modules that included review of IASLC scoring criteria and training using the web-based calculator using 30 imaged slides with post-training discussion to discuss discordant cases
- Data entered into major pathologic response (MPR) calculator that included tumor bed dimensions, % viable tumor, % necrosis
    - Pre-embedded formulas calculated weighted and unweighted averages of viable tumor on all slides; weighted average took into account variable proportions of tumor bed on each slide
    - Interobserver reproducibility based upon MPR scores

Results:

- 84 NSCLC samples reviewed (n = 45 adenocarcinomas, n = 27 squamous cell carcinomas, n = 12 other NSCLC)
- Highest concordance seen at the extremes of >95% viable tumor and 0% viable tumor; least concordance when viability between 30 and 60% (Figure 2)
- No significant difference between unweighted and weighted methods
- 16 cases considered as having discordant interpretations
    - Reasons for discordance summarized in Table 1

Take-home message: The IASLC criteria for assessing pathologic response in post-neoadjuvant lung cancers can be reliably applied by experts using a MPR calculator (not “eyeballing” as I suspect many do), particularly when there is excellent or poor response to therapy, but delineation of the tumor bed in the settings of apical cap or pleural fibrosis and marked stromal inflammation represent the greatest potential problems in interpretation.

Articles for notation

Neoplastic lung disease

Cho IS et al. Clinical implication of the 2020 international association for the study of lung cancer histologic grading of surgically resected pathologic stage 1 lung adenocarcinomas: prognostic value and association with computed tomography characteristics. Lung Cancer 2023; 184:107345.

Take-home message: The aim of this study was to assess the performance of histologic subtyping for the stratification of $n = 356$ stage 1 non-mucinous lung adenocarcinomas, using the conventional grading system compared to the updated grading system, the latter of which assigns a higher grade to cases in which there is a greater percentage of high-grade patterns. Performance was based upon postoperative recurrence. Approximately 24% of cases were re-classified from intermediate grade to grade 3 using the updated system with higher recurrence rate in reclassified tumors, suggesting that the new system has greater prognostic value.

Fakhri NL et al. Desmoplastic small round cell tumor involving serous fluid: cytologic features and diagnostic pitfalls: a series of 8 cases. Am J Clin Pathol 2023; 160:417-424.

Take-home message: This study is helpful for pathologists who might review pleural fluids (like us!) and hence its inclusion here. The authors detail the cytomorphology of metastatic desmoplastic small round cell tumor (DSRCT) in 5 pleural and 4 ascitic fluid samples, describing varied patterns that represent a diagnostic pitfall particularly in cases in which the diagnosis is not already known or expected. In some cases, the findings mimic the appearance of carcinoma or mesothelioma; a problem further compounded by the fact that these tumors frequently express MOC-31 and claudin-4, so in young patients do not forget about DSRCT and perform gene fusion for EWSR1-WT1 if needed.

Goto E et al. Stepwise progression of invasive mucinous adenocarcinoma based on radiological and biological characteristics. Lung Cancer 2023; 184:107348.

Take-home message: The clinical and biologic characteristics of 70 invasive mucinous adenocarcinomas were detailed, comparing 38 solitary tumors to 32 pneumonic-type tumors. There was no difference in clinical parameters (age, sex, smoker status) or genetic alterations between the cohorts, but MUC6 expression was more common in the solitary type, while MUC1 was more frequent in the pneumonic-type. Solitary tumors with radiographic groundglass opacity had the best prognosis (5-year OS 95.8%), while pneumonic-type with crazy-paving appearance on chest CT had the worst prognosis (5-year OS 50.0%).

Ikeda H et al. Immunologic significance of CD80/CD86 or major histocompatibility complex-II expression in thymic epithelial tumors. JTO Clin Res Rep 2023; 4:100573.

Take-home message: Eighty-six thymic epithelial tumors were evaluated for low or high expression of CD80, CD86, MHC-II, and MHC-I by immunohistochemistry. Epithelial cells of type B3 thymomas had the highest expression of CD80/CD86, while MHC-I and MHC-II did not differ between subtypes, and perhaps not surprisingly, thymic carcinomas and types A and B3

thymomas had low T-cell infiltration. The authors then developed CD80-expressing and MHC-expressing mouse tumor cells lines, both of which showed a dramatic response to anti-PD-L1 monoclonal antibody, suggesting that CD80/CD86 and MHC-II might be useful tools in predicting response of thymic epithelial tumors to immune checkpoint inhibitors.

Klein S et al. Intratumoral abundance of M2-macrophages is associated with unfavorable prognosis and markers of T-cell exhaustion in small cell lung cancer patients. Mod Pathol 2023; 36:100272.

Take-home message: In this study, the composition of the tumor microenvironment was interrogated in 45 biopsy samples of SCLC, using whole-slide-images and deep-learning to evaluate the prognostic relevance of different immune cell types to overall survival. The authors discovered that "high" numbers (exact range unclear to me) of intratumoral M2 macrophages, characterized by positive staining for CD163 and CD204, was associated with worse OS, even when controlling for T, N, and M status. The human scoring the infiltrate as CD163-high vs. CD163-low was comparable to the computer.

Tanaka T et al. The prognostic impact of a high number of peritumoral alveolar macrophages in neuroendocrine carcinoma in the lung. Pathol Int 2023; 73:497-508.

Take-home message: In this work, the authors methodically counted numbers of alveolar macrophages (AM) located around (peritumoral [pAM]) and apart (distant [dAM]) from 73 neuroendocrine carcinomas, including 36 SCLC/combined SCLC and 37 LCNEC, as well as 29 carcinoid tumors (10 typical; 19 atypical), to assess impact on prognosis. Neuroendocrine carcinomas typically had more pAM than carcinoid tumors, and LCNCs had the most of any group. The number of pAMs correlated with increased risk of recurrence in neuroendocrine carcinomas; however, so did tumor size >3 cm, presence of lymph node metastases, and need for adjuvant therapy.

Yeung V et al. High levels of expression of Trop-2 in thymic epithelial tumors. Lung Cancer 2023; 184:107324

Take-home message: Here, the authors assess immunohistochemical expression of trophoblastic antigen (Trop-2) and PD-L1 in thymic epithelial tumor samples, including 17 thymomas and 12 thymic carcinomas, and 13 samples of normal thymus. Normal thymic tissue showed no to 1+ (weak) expression of Trop-2, while most (n = 13) thymomas showed moderate (2+) to strong (3+) expression and all thymic carcinomas were weakly to strongly positive; there no statistically significant relationship between PD-L1 and Trop-2 expression. These findings suggest that Trop-2 might be a potential target for drugs conjugated to anti-Trop-2 antibodies.

Non-neoplastic lung disease

Das S et al. New autopsy technique in COVID-19 positive dead bodies: opening the thoracic cavity with an outlook to reduce aerosol spread. J Clin Pathol 2023; 76:664-670.

Take-home message: The authors detail an abdominal approach to the thoracic organ dissection that may be applied to all autopsies in which decedents are suspected of having lung

infections by respiratory pathogens. The goal of this technique is to minimize aerosol droplet spread.

Case reports

Li H et al. A giant dendritic fibromyxolipoma in the right thorax – a rare entity. Chest 2023; 164:e89-e91.

Take-home message: This case report documents an intrathoracic dendritic fibromyxolipoma in an 11-year-old female and includes a brief review of the clinical and pathologic features of this entity, including differential diagnosis.

Self A et al. An unexpected cause of lung disease identified after lung transplantation. Chest 2023; 164:e111-e115.

Take-home message: A 54-year-old woman with systemic lupus erythematosus underwent lung transplant for interstitial lung disease. The radiographic findings were described as lower-lobe predominant fibrosis with honeycombing and prominent septal thickening and reticulation. While the authors describe the explanted lungs as having interstitial fibrosis, honeycombing, and fibroblastic foci (sounds like usual interstitial pneumonia to me!), they conclude that they patient has diffuse idiopathic pulmonary neuroendocrine cell hyperplasia due to the presence of multiple foci of neuroendocrine cell hyperplasia and carcinoid tumorlets.