Discussion articles

Articles for notation
Neoplastic diseases


**Discussion articles**

**Purpose:** To determine whether there are morphologic or immunohistochemical findings that would allow us to identify those allograft biopsies with anti-HLA antibody–mediated lung injury.

**Methods:**
- study population: 23 patients who developed anti-HLA antibodies and clinical allograft dysfunction
- control group: 26 patients matched for age, sex, and postoperative day, with similar grades of acute cellular rejection without anti-HLA antibodies
- Morphologic features that were studied included: acute cellular rejection grades (A and B scores); endothelialitis, capillaritis, acute lung injury, eosinophils, large airway inflammation, plasma cells, increased alveolar septal neutrophils, and C4d deposits (by IHC).

**Results:**
- Of the 23 patients, 17 had coexistent high-grade acute cellular rejection, 5 had patchy acute lung injury, and 1 had bronchiolitis
- When the 17 cases of acute cellular rejection with coexistent anti-HLA antibodies were compared with a matched group of 26 patients with equivalent cellular rejection grade without anti-HLA antibodies:
  - The only morphologic feature that separated the 2 groups was capillaritis seen in a minority of cases (18%).
  - C4d deposits were seen in both groups, although more frequently in those cases with anti-HLA antibodies (76% vs. 24%).
  - There is no difference in the incidence of endothelialitis, large and small airway inflammation, neutrophil margination and the presence of eosinophils and plasma cells

**Take home points:**
- The development of anti-HLA alloantibodies often occurs in the setting of acute cellular rejection.
- Capillaritis was seen in 4/22 (18%) cases, usually in the context of cellular rejection.
- C4d staining does not consistently separate the 2 groups (with or without anti-HLA alloantibodies), although more commonly seen in patient with anti-HLA alloantibodies.
Purpose: To determine the concordance among RT-PCR, IHC, and FISH in determining ALK mutation status.

Methods: 46 cases of formalin fixed, paraffin embedded tissue with unknown ALK status were tested; this set was mostly patient who lacked an EGFR mutation.
- IHC for ALK (CD246) was performed using a Dako mouse monoclonal antibody; three viewers scored independently to determine interobserver variability
- FISH was performed using the LSI ALK Dual Color break-apart rearrangement probe from Abbott; three viewers independently scored the FISH to determine interobserver variability
- RT-PCR was performed using total RNA extracted from the tissue followed by analyzation

Results:
- By RT-PCR: EML4-ALK fusions detected in 24%
- By FISH: unanimous positive assessment in 15% of cases; agreement between scorers was excellent for the 3a/b mutation but poor for the variant 1 mutation (only 1 of 9 samples positive by RT-PCR for variant 1 was positive on FISH)
- By IHC: detected in 20% of cases; agreement was 100% between scorers
  - Only 2 of 9 IHC positive cases were RT-PCR and FISH positive
- Concordance between all three methodologies was 100% for variant 3a/b but NO concordance for variant 1
- 80% agreement between RT-PCR and FISH; 70% between RT-PCR and IHC; and 67% between FISH and IHC

Take home points:
- IHC is cheap and easy but lacks the sensitivity of other methodologies
- FISH requires specialized equipment and expertise to read it out and isn’t as sensitive as RT-PCR
- RT-PCR is the most sensitive (the authors seem to advocate for this method)
- The different variants of the EML4-ALK fusion have different sensitivities.

- **Purpose:** To investigate the prognostic impact of mutations in TP53, KRAS, or EGFR in resected, early-stage non-small cell lung cancer and to evaluate their use as a biomarker of disease progression.

- **Methods:** The group used the European Early Lung Cancer (EUELC) project, which is a collaboration involving 22 centers throughout Europe, which has recruited a total of 762 patients with surgically resected primary lung cancers who were considered at high risk of developing a second cancer or a metastasis.
  - Patients who developed a recurrence or metastasis or who died of disease were considered progressive disease; patients who were alive, asymptomatic, or who were not undergoing treatment at the time of last follow-up were considered disease-free.
  - A total of 250 patients with frozen tissue suitable for DNA analysis as well as known follow-up status were selected for TP53 and KRAS analysis (EGFR was only done in adenocarcinomas)
    - Includes 11 never smokers, 86 former smokers, 152 current smokers
    - Includes 110 squamous cell, 133 adenocarcinomas
    - TP53 analyzed using HPLC followed by PCR and sequencing
    - KRAS and EGFR analyzed using PCR and sequencing
    - P53 immunohistochemistry also performed, and evaluated for percent positive and intensity of staining, which was combined into a composite score.
  - Statistical analysis was performed to find association between markers and clinical features as well as between markers

- **Results:** Of tumors analyzed: 48.4% had TP53 mutations, 18.5% had KRAS mutations, and 13.1% had EGFR.
  - 18 had mutations in two genes (11 with PT53 + KRAS, 6 with TP53 + EGFR and 1 with KRAS + a silent EGFR mutation)
  - TP53 mutations were less frequent in adenocarcinoma than squamous cell carcinoma, and KRAS mutations were found preferentially in adenocarcinoma than squamous cell.
  - Neither smoking or asbestos exposure was associated with TP53 or KRAS mutation status though KRAS mutations were more common in ever smokers than never/former smokers and never smoking status was significantly associated with EGFR mutations
  - Strong association between TP53 mutation and p53 overexpression (though 25% of tumors with wild type TP53 also had p53 overexpression).
  - NO association with any of the genes and prognosis (nor with p53 immuno)
    - Analyzed a subgroup of more “homogenized” patients from one institution—no impact on prognosis in that group either.

- **Take home points:** This work served to confirm much of what was already known about molecular derivations in NSCLC—namely that TP53 mutations are more common in SCC and KRAS more common in adeno.
  - None of these mutations have any prognostic significance in this study.
- Previous Japanese reports have found EGFR mutations to be associated with a better prognosis, but the authors surmise this may be due to population differences.
- The authors also surmise that TP53 and KRAS mutations may represent very early events in lung carcinogenesis, and that tumor behavior may actually depend more on additional events caused by tobacco carcinogens.

- **Purpose:** To determine whether lymphovascular invasion (LVI) increases the risk of local or distant metastases and to investigate the association between LVI in the primary tumor and the risk of lymph node metastases.

- **Methods:** Identified all patients who underwent surgery for T1-3N0-2 NSCLC at a single institution between 1995-2008 (patients with neoadjuvant chemotherapy or who had not had lymph nodes resected were excluded).
  - All H&E slides interpreted by a single thoracic pathologist (though only at the time of resection, NOT at the time of this study) in a “vast majority of cases”, and LVI was considered tumor cells within lymphatic channels, veins/venules, and/or arteries/arterioles.
  - A local failure (LF) was defined as disease recurring at resection margin or regional lymph nodes.
  - Other factors measured with overall survival and development of distant metastases (DM).

- **Results:** 1529 patients met inclusion criteria; LVI was seen in 23%
  - Factors associated with regional LN involvement included: LVI, increasing size of the mass, lobar resection (vs. sublobar), thoracotomy (vs. VATS), visceral pleural invasion, and squamous/large cell histology
    - LVI also associated with an increased number of positive lymph nodes
  - LVI associated with a statistically significant increased risk of LF on univariate analysis only (disappeared on multivariate)
  - LVI was significantly associated with worse freedom from DM
  - When analyzed according to histology, LVI association with increased risk of DM and death was only found in adenocarcinomas.
  - LVI also associated with increased risk of death

- **Take home points:** In the current state of practice, LVI does NOT impact treatment recommendations in NSCLC, however in this study LVI was associated with nodal metastases and therefore may be useful in determining the need for adjuvant chemo/radiation in patients who do not undergo lymph node sampling or who undergo a VATS procedure (which may lead to a less thorough node sampling).
  - They put a lot of pressure on diagnosing LVI on small biopsy

- **Purpose**: To study the functional significance of astrocyte-elevated gene-1 (AEG-1) in non–small cell lung cancer.
- **Methods**: Study the AEG-1 mRNA and protein levels in non–small cell lung cancer cell lines and tissues; ectopic expression or small interfering RNA silencing of AEG-1; subcutaneous xenografts of non–small cell lung cancer cells engineered to express AEG-1; retrospective correlation of survival time and AEG-1 expression level.
- **Results**: Up-regulation of AEG-1 was observed in non–small cell lung cancer cell lines and tissues; ectopic expression or small interfering RNA silencing of AEG-1 markedly enhanced or inhibited the invasive ability of non–small cell lung cancer cells, respectively. Subcutaneous xenografts of non–small cell lung cancer cells engineered to express astrocyte-elevated gene-1 were highly invasive compared with the parental cells. The overall survival time in patients with high AEG-1 expression was notably shorter than that in patients with low AEG-1 expression.
- **Take-home message**: AEG-1 plays a crucial role in the carcinogenesis and aggressiveness of non–small cell lung cancer, and high AEG-1 level leads to a poor clinical prognosis.


- **Purpose**: To assess the individual diagnostic value of histopathology, imprint cytology and brushing cytology for the diagnosis of malignancy during flexible bronchoscopy.
- **Methods**: The authors performed the three sampling techniques in 102 consecutive patients with suspected pulmonary pathologies and compared the definitive diagnosis with those of the three sampling techniques for their diagnostic values.
- **Results**: 33.3% of histopathological specimens, 31.4% of imprints and 26.5% of brush biopsy specimens were positive for malignancy. The values for sensitivities were 94% for histopathology, 89% for imprint cytology and 75% for brushing cytology, respectively. Although brushing cytology had limited sensitivity, in two cases a malignant lung tumor was only diagnosed from cytological examination of brushing.
- **Take-home message**: Routine imprint cytology does not increase the diagnostic sensitivity, whereas routine brushing cytology should be used in combination with histopathology to obtain the highest diagnostic rate of yield.

• **Purpose:** To evaluate the significance of FGFR1 in lung cancer.

• **Methods:** Tissue microarrays were constructed containing 380 lung cancer samples including squamous cell carcinomas (SCC), adenocarcinomas (ADC), non-small cell lung cancer not otherwise specified, metastases, neuroendocrine tumors, large cell lung cancer and small cell lung cancer. FGFR1 expression was scored by IHC, and copy number was determined by FISH.

• **Results:** High expression of FGFR1 was associated with increased FGFR1 gene copy numbers in squamous cell carcinoma. There were no significant associations between FGFR1 and clinicopathological parameters (age, sex, stage and grade). 14 out of 133 SCC cases (10.5 %) showed ≥4 copies of the FGFR1 gene while the frequency was considerably lower for lung ADC (3 out of 64 cases, 4.7 %). This difference, however, failed to reach statistical significance. Other interesting trends without statistical significance: SCC patients with FGFR1 copy number increases carried a worse prognosis with survival; ADC with increased FGFR1 signals showed a better survival than the amplified SCCs.

• **Take home message:** FGFR1 overexpression is more common in SCC; FGFR1 expression or gene copy number alterations, in general, did not show any significant association with survival.


• **Purpose:** To evaluate the utility of immunohistochemical staining for glucose transporter 1 (GLUT-1), a protein typically found on erythrocytes and aberrantly expressed in malignant mesothelial cells, in diagnosing malignant mesothelioma in the pleura and peritoneum.

• **Methods:** Studied 30 thoracic mesotheliomas (21 epithelioid, 9 sarcomatoid/biphasic), 23 thoracic benign mesothelial hyperplasia, 15 thoracic fibrosing pleuritis, 135 abdominal mesotheliomas (100 epithelioid, 35 sarcomatoid/biphasic), 3 abdominal well-differentiated papillary mesotheliomas, 18 abdominal reactive mesothelial hyperplasias—stained whole slides with GLUT-1. Positive = minimum of 5% cells staining positively.

• **Results:** GLUT-1 positive in 15/30 thoracic malignant mesos (8/21 epithelioid, 7/9 biphasic/sarcomatoid) and 73/135 peritoneal malignant mesos (51/100 epithelioid, 22/35 biphasic/sarcomatoid). Overall Sensitivity 53% and specificity 98%.

• **Take home message:** Seperating malignant mesothelioma from reactive mesothelial hyperplasia remains an H&E diagnosis (but this marker may help out if you want to try it). The article does contain a nice review of studies looking at other potentially helpful markers in the diagnosis.


• **Purpose:** To try to identify a large number of volatile organic compound (VOC) fingerprints and try to distinguish them from one another in order to develop an air-sampling method that may be of use in so-called “breathomic” diagnostic evaluation of patients.
Methods: 30 patients total, including 20 patients strongly suspected of having cancer, underwent breath collection consisting of breathing into a tedlar bag which is designed to catch deep lung volumes and also through a bronchoscopic apparatus that was adapted to collect breath through an endoscope. These samples then were submitted to an artificial olfactory system, which is a gas sensor array. A mass spec fingerprint was also determined. Lots of complicated statistics were performed, including one appropriately called a “confusion plot”.

Results: The technology was able to determine differences between patient without cancer, those with adenocarcinoma, and those with squamous cell carcinoma. They did not attempt to identify the VOC compounds composing these fingerprints.

Take home points: Although probably still a distant possibility, breath sampling remains a diagnostic possibility in diagnosing lung cancer.


Purpose: Case series of five micronodular thymic carcinomas with lymphoid hyperplasia.

Methods: Clinicopathologic observation of 5 cases of thymic carcinoma with a micronodular growth pattern and prominent lymphoid hyperplasia.

Results: Morphologic features included small tumor nodules separated by lymphoid stroma; tumor cells were large, round-to-oval, with abundant eosinophilic cytoplasm in three cases while the remaining two had spindled cells. Four of the five patients were alive at 3-26 months following diagnosis; one patient died of disease. The authors state that these cases differ from thymomas due to overt signs of malignancy (atypia, mitotic activity, comedonecrosis).

Take home points: The micronodular growth pattern is an unusual pattern seen in thymic carcinoma with features very similar to the micronodular thymoma with lymphoid hyperplasia. Important differential diagnoses include metastatic carcinoma or sarcoma involving mediastinal lymph nodes.


Purpose: Case series and review of immunoprofile, electron microscopic features of pleomorphic mesothelioma, which is included in the WHO classification as a subtype of epithelioid mesothelioma.

Methods: Ten cases of pleomorphic mesothelioma were analyzed with H&E sections, immunohistochemistry, and electron microscopy.

Results: All patients were men (age 61-74); asbestos exposure in 7; smoking history in 5. Nine cases were pleural and one was peritoneal. Follow-up data available in seven—six died, one alive with recurrent disease at 3 months. On H&E tumors were composed of pleomorphic epithelioid cells in varying proportions, with irregular nuclei often containing large nucleoli. All cases had high mitotic counts. Immunohistochemically, all cases were strongly positive for pancytokeratin and cytokeratin 7; most were also positive for CK5/6. Other mesothelial markers (calretinin, WT1, mesothelin, podoplanin) were variable. All
cases were negative for MOC31, CEA, CD15, TAG72, and TTF1. Electron microscopically, all cases demonstrated characteristic microvilli.

- **Take home points:** Pleomorphic mesotheliomas maintain the immunophenotype seen in other epithelioid mesotheliomas, which can be helpful in distinguishing this tumor from pleomorphic carcinomas of the lung. The author also advocates for keeping this subtype of mesothelioma in the category of “epithelioid” rather than “sarcomatoid”.


- **Purpose:** To evaluate the prognostic factors and patterns of recurrence in resected node-negative nonsmall cell lung cancer (NSCLC) with visceral pleural invasion (VPI), including PL1 (invasion of the tumor beyond the elastic layer) and PL2 (invasion to the visceral pleural surface).
- **Methods:** 355 patients with resected node-negative NSCLC with VPI, including PL1 in 300 patients (84.5%) and PL2 in 55 (15.5%), were followed (median follow-up 54.2 months) for recurrence.
- **Results:** The 5-year overall survival rate and probability of freedom from recurrence were 61.9% and 66.2%, respectively. 107 patients (30.1%) developed recurrence, including local recurrence only in 20 patients (18.7%), distant metastasis only in 59 (55.1%), and both local recurrence and distant metastasis in 28 (26.2%). Thirteen of the 107 patients (12.1%) with recurrence developed malignant pleural effusion. The percentage of malignant pleural effusion in the PL2 group was significantly higher than that in the PL1 group. Patients with PL2 had significantly worse overall survival and lower probability of freedom from recurrence in multivariate analysis.
- **Take home message:** PL2 was a significant prognostic factor for recurrence and worse overall survival in node-negative NSCLC with VPI.


- **Purpose:** To characterize the two cell types (polygonal and cuboidal) composing pulmonary sclerosing hemangioma (PSH).
- **Methods:** 45 PSH specimens were examined for expressions of β-catenin, Axin, and C-myc by immunostaining. The two cell types were captured by laser capture microdissection from 28 PSH specimens, and total RNA was extracted. Messenger RNA (mRNA) expression of Axin and C-myc was examined by reverse transcription polymerase chain reaction (RT-PCR).
- **Results:** By immunostaining, β-catenin was predominantly strongly expressed on the cell membrane of cuboidal cells, while in polygonal cells, β-catenin was predominantly expressed in the cytoplasm. Axin was expressed in cuboidal cells in 93 % of our 45 cases, but only expressed in 18 % of these in polygonal cells. C-myc expression in polygonal cells was significantly stronger than in cuboidal cells. RT-PCR confirmed that expression level of Axin
mRNA was higher in cuboidal cells and expression level of C-myc mRNA was higher in polygonal cells.

- **Take home message:** The two PHS cell types have distinct expression of β-catenin, Axin, and C-myc, suggesting that their differentiation status may be different. The higher expression of C-myc in polygonal cells suggests that these cells might have higher proliferative activity.


- **Purpose:** To present the clinicopathologic and immunohistochemical features of 65 primary thymic carcinomas.
- **Methods:** Cases meeting classification for thymic carcinoma per WHO criteria who had undergone total thymectomy were studied. Cases were staged using the Masaoka system, and were stained for panCK, p63, CK5/6, CK7, FoxN1, Pax8, c-kit, CD5, CDX2, CD205, calretinin, TTF1, napsin, CD30, and Ki-67 if a block was available.
- **Results:** Tumors were classified as follows: 38 squamous cell carcinomas (3 basaloid), 12 high-grade undifferentiated carcinomas, 4 lymphoepithelioma-like carcinomas, 3 spindle cell carcinomas, 2 mucinous adenocarcinomas, 2 papillary carcinomas, 2 clear cell carcinomas, 1 rhabdoid carcinomas, and 1 anaplastic carcinomas. Immunohistochemically, All tumors were positive for panCK; all other markers showed variable reactivity—81% p63, 77% CK5/6 and Pax8, 68% CK7 and FoxN1, 65% c-kit, 39% CD5, 10% calretinin and CD205, 2% CDX2. No tumors were positive for TTF1, napsin, or CD30. Applying the Masaoka staging system did not accurately predict outcomes.
- **Take home points:** Serves as an overview of thymic carcinomas. The most interesting part of this study for me is the Pax8 positivity—an often used marker in the gyn/gu differential diagnoses.


- **Purpose:** To introduce a new 3-tiered TNM-derived staging system that shows better correlation with patient outcomes than the Masaoka system.
- **Methods:** Included cases with a diagnosis of primary thymic carcinoma with material available from thymectomy and in which a lymph node dissection was performed at the same time. They devised a staging system and the assigned each case to a stage and performed survival analysis. The proposed system is as follows:
• Results: There were statistically significant differences between the three stages using this staging system.

• Take home points: A staging system for thymic carcinomas has not been agreed upon in the literature, and currently staging for this neoplasm is lumped in with thymomas. This system does seem a lot easier to use than the Masaoka system—anything that does not require me to find capsular invasion in the thymus is alright by me.


• Purpose: Tumors larger than 5cm without lymph-node invasion, were shifted from stage IB (6th TNM) to stage II (7th TNM). The aim of this analysis was to assess the rate of patients who shifted from stage IB (6th TNM) to stage IIA/B (7th TNM), and to describe specific characteristics, clinical outcome, and putative prognostic factors of this subpopulation.

• Methods: 467 patients who underwent radical surgery for primary 6th TNM-T2N0 non–small cell lung cancer. Categorical variables (age, sex, smoking status, type of surgery, grading, and histological type) were cross-tabulated by tumor staging according to the 7th TNM edition, and they were tested for association with stage and survival.

• Results: 118 patients shifted to stage II, mainly older patients and patients with a sarcomatoid or a poorly differentiated carcinoma. Median overall survival time was significantly different across stages. Among the factors investigated, only the tumor dimension resulted in being statistically significant in multivariate analysis.

• Take-home messages: Nearly a quarter of patients shifted from stage I (6th TNM) to stage II (7th TNM), mainly older patients and patients with a sarcomatoid or a poorly differentiated carcinoma. Tumor dimension is the single most important prognostic factor.

• **Purpose:** To try to correlate the maximum standardized uptake value (SUVmax) on PET scans with tumor histology in patients with malignant mesothelioma.

• **Methods:** Studied 71 patients with a diagnosis of malignant pleural mesothelioma who underwent PET scanning without prior treatment with chemotherapy. Tumors were separated into “low SUVmax” and “high SUVmax”. All tumors were reviewed by pathologists and classified according to WHO criteria. Epithelioid mesothelioma patterns (trabecular, tubulopapillary, micropapillary, solid, pleomorphic) were recorded in 5% increments (wonder who their pathologist was…). Mitoses were also counted so as to correlate with SUVmax as well. They did not exclude patients who had undergone a pleurodesis.

• **Results:** Mesotheliomas with a nonepithelioid phenotype had a statistically significantly higher SUVmax than those with an epithelioid phenotype. There are a lot of statistics included, but there were no other statistically significant findings.

• **Take home points:** Doesn’t look like radiology will be displacing histologic examination anytime soon as higher SUVmax did not correlate with overall survival.


• **Purpose:** To evaluate the significance of podoplanin-positive cancer-associated fibroblasts and CD204-positive tumor-associated macrophages as risk factors for recurrence in patients with stage I lung adenocarcinoma.

• **Methods:** Studied a total of 304 patients who underwent surgical resection of stage I lung adenocarcinoma at a single institution. Tumors evaluated, typed, staged, based on H&E. Immunostains for CD204 and podoplanin were performed and fibroblasts were evaluated for podoplanin (considered positive if >10% CAF positive), macrophages for CD204 (evaluated as number CD204+ TAM/400x field and then separated into high and low groups).

• **Results:** Podoplanin-positive CAFs and CD204-positive TAMs were independent predictors of recurrence in both univariate and multivariate analyses.

• **Take home points:** The tumor microenvironment may contribute to the proliferation, invasion, and metastases of tumor cells. As these markers were associated with increased risk of recurrence, perhaps in the future they can be used to identify patients at risk of relapse and therefore help guide adjuvant chemotherapy in these patients.

Non-neoplastic diseases


• **Purpose:** To characterize the relationship of mast cells to fibrotic lung diseases.

• **Methods:** Lung tissues from patients with idiopathic pulmonary fibrosis (IPF), chronic hypersensitivity pneumonitis (HP), systemic sclerosis (SSc)-related interstitial lung disease (ILD) and normal individuals were subjected to chymase immunostaining and the mast cell
density was quantified. Eosinophils were quantified by immunostaining for eosinophil peroxidase. Changes in lung function were correlated with mast cell density.

- **Results**: Lung tissue from IPF patients had a higher density of mast cells than that from patients with HP, SSc-related ILD or normal lungs. IPF lung tissue had a higher density of eosinophils than normal lung, but there was no correlation between mast cell density and eosinophil density in IPF lung. IPF patients with high mast cell density (median and above) had a slower rate of decline in forced vital capacity (FVC) than IPF patients with low mast cell density (below median).

- **Take home message**: Mast cell density in IPF lungs is higher than in other fibrotic lung diseases and normal lungs. Increased mast cell density in IPF may predict slower disease progression.


- **Purpose**: To see if postmortem CT scans with image-guided biopsies can be as accurate at determining cause of death as a full forensic autopsy in cases of sudden death.

- **Methods**: Twenty patients who had antemortem reports of chest pain and who subsequently died suddenly and were scheduled for autopsy underwent postmortem CT angiography with image guided biopsies targeted at areas of interest as determined by the CT scan. Biopsies included samples of the heart, lungs, and suspected emboli. This was then compared to the full autopsy with standard histologic specimens taken.

- **Results**: In 19 of 20 patients the findings in the postmortem CT angiography scans validated those findings seen at full autopsy. In five cases, the image findings led to a change in the autopsy procedure. In one case, the imaging with selected biopsies missed early infarction of the papillary muscles as this area was not included in the biopsy protocol.

- **Take home points**: Seems like an awfully expensive replacement for a simple autopsy, but is still a fascinating alternative that sticks radiology with the sometimes expensive and unsavory task of staffing autopsies. Not ready for prime time (and probably will never be ready for prime time) but still curious nonetheless!