PULMONARY PATHOLOGY JOURNAL CLUB
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Nonneoplastic diseases
Discussion articles


- **Purpose:** Nodular pulmonary amyloidosis (NPA) often is an incidental finding on imaging and is limited to the lung; these have been regarded as almost always AL-type amyloid, and a small number of cases have reported an associated low-grade B-cell lymphoma but it remains controversial whether ALL cases of NPA related to lymphoma. This study sought to elucidate the underlying mechanisms of NPA.

- **Methods:** In house and consultation archived at a single institution (Mayo) were searched for NPA.
  - IHC: CD3, CD19, CD20, kappa and lambda Ig light chains, serum amyloid P component, serum amyloid A, transthyretin, and Ig heavy chains performed on all cases; Ig J chain performed in selected cases.
  - LL MS/MS performed on peptides that were laser-microdissected from amyloid taken from FFPE tissue, and the proteomic composition of the amyloid determined.
  - In 12 cases, genomic DNA was extracted for PCR to assess for clonal Ig gene rearrangements.
  - FISH was conducted on 14 cases for MALT1 gene locus.

- **Results:** 18 NPA cases identified
  - 5/14 (36%; not sure why only 14--were these the monotypic cases only?) had an underlying connective tissue disorder, and two of these patients also had concurrent LIP
  - All cases demonstrated nodular amyloid deposition with lymphoplasmacytic infiltrates.
    - 14/18 the plasma cells were monotypic, all of which were positive for CD19.
    - The lymphoid nodules appeared to be reactive follicles, not clonal
    - The monotypic plasma cells were concordant by IHC and LC MS/MS
  - 6/12 cases had a clonal Ig gene rearrangement by PCR; no cases had a MALT1 translocation
  - All cases demonstrated Ig-associated amyloid on LC MS/MS (12=kappa; 4=lambda; 2=both)
    - 13 (72%) also had significant codeposition of heavy chains--a higher frequency than this occurs in patients with systemic amyloidosis.
  - 3/12 patients had a monoclonal serum protein, two of which had a distinct heavy chain component than that in the serum and one of whom had a lymphoplasmacytic lymphoma.
  - 14 patients had follow up data: no patient developed systemic amyloidosis, 3 had recurrence of NPA, 2 had recurrence PLUS cutaneous MALT lymphoma (one of which had associated amyloidosis that was identical to the lung amyloidosis)

- **Take home points:**
  - In most cases, the amyloid results from localized production of clonal Igs.
  - In NPA, kappa predominates over lambda, in contrast to systemic amyloidosis, and NPA shows codeposition of heavy chains.
The plasma cells were CD19 positive, a finding which is associated with lymphoplasmacytic neoplasms.

One third of patients had a history of connective tissue disorders, possibly suggesting a triad of NPA, CTD, and MALT lymphoma. Therefore the authors suggest that NPA represents a lymphoplasmacytic neoplasm in the spectrum of MALT lymphoma.

33% of patients had recurrence.

Two patients had polytypic light chains (equal kappa and lambda) and these patients did not have recurrence of disease.

- **Purpose:** Identifying invasion in adenocarcinomas with a predominant lepidic growth pattern may be difficult, especially when biopsy-site changes are introduced. This group sought to determine whether biopsy-related changes occur in the lung, and whether they can potentially cause difficulties in identifying invasion.

- **Methods:** All core needle biopsy specimens showing a well-differentiated adenocarcinoma with a lepidic pattern along with subsequent resection specimens were studied ("moderate" were excluded so as to only study cases in which the invasive component would have an impact however no criteria for "well" vs. "moderate" given)
  - Cases included AIS, MIA, and well-differentiated adenocarcinoma with a predominant lepidic pattern as defined by IASLC/ATS/ERS proposed classification. The authors describe their diagnostic criteria in the last paragraph of page 443.
  - The presence of biopsy-site change was noted and described, including appearance, size, proximity to tumor, and presence of entrapped epithelium, as was time interval since biopsy.

- **Results:** 26 resected adenocarcinomas (14=MIA, 2=invasive well-diff, 10 AIS) summarized in Table 1 on page 444.
  - Biopsy site changes identified in 9 (35%)
  - Mean time between biopsy and resection 26.2 days
  - Biopsy site described as a linear scar composed of collagen and plump fibroblasts with variable inflammatory cells; pigment-laden macrophages present in 4 and FBGCs in 3
    - Biopsy site present within tumor in 5 cases and "in close proximity" in other 4
  - Benign entrapped lung epithelium in all 9, and 4 entrapped malignant epithelium, which "closely mimicked stromal invasion" in these cases
• **Take home points:**
  - Biopsy site changes are important to recognized because of their resemblance to desmoplastic stroma.
  - The distinction of AIS from MIA as well as accurately measuring the size of an invasive focus is going to start mattering and this will make things even MORE difficult.
  - Biopsy scars are distinguished by their low-mag appearance, which looks linear like a needle tract...and you can also confuse it for organizing pneumonia (although in theory this should have little clinical consequence).

- **Purpose:** FISH-based analysis has become the standard for the diagnosis of ALK-rearranged lung carcinomas, and the ALK break apart FISH probe kit from Abbott has become the FDA-approved companion diagnostic for crizotinib therapy. However, FISH is time-consuming and expensive, and can also be prone to false-positives and false-negatives as well as interobserver variability. IHC with a new ALK clone, 5A4, has shown promise, so this group compared ALK FISH vs. IHC from cases derived from their workflow, and also examined the basis for any discrepancies.

- **Methods:** ALK FISH performed using the Abbott dual color, break-apart rearrangement probe, and classified as positive when 15% or more nuclei showed split signals. IHC performed using clone 5A4 from Novocastra. EGFR and KRAS mutations were also analyzed using PCR followed by sequencing.

- **Results:** 830 cases underwent ALK FISH testing during the time period (9/2010 - 4/2012), 25 (3%) of which were positive; 186 of these cases were tested for ALK by IHC
  - IHC expression detected in 13 cases, negative in 170 cases; three cases had significant intratumoral heterogeneity.
  - 12 IHC-positive cases were also FISH positive
    - 1 IHC positive case was FISH negative on initial review
    - 2 IHC negative cases were FISH positive
  - **False-positive FISH cases:** two had translocations in some proportion of cells and one of these was found to have an atypical ALK rearrangement (Table 1; page 325)
  - **False-negative FISH cases:** rereview of the original FISH material demonstrated that counts had been performed on areas of pneumocyte hyperplasia and it was actually positive
  - **False-negative IHC:** low-volume specimen

<table>
<thead>
<tr>
<th>ALK FISH</th>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>Negative</td>
<td>1</td>
<td>162</td>
<td>163</td>
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<tr>
<td>Low (1+)</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>High (2+)</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total positive</td>
<td>13</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>162</td>
<td>176</td>
</tr>
</tbody>
</table>

*Total case tally does not include 10 cases that were insufficient either by FISH or IHC. ALK, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization.*

- **Take home points:**
  - ALK FISH, although considered the gold standard for clinical testing, can have both false-positive and false-negative results.
  - ALK IHC is generally considered less sensitive, although analysis of discrepant cases in this series revealed this may not be entirely true.
  - This group advocates a combined FISH *and* IHC approach to maximize both sensitivity and specificity—they include their diagnostic algorithm on **Figure 3, page 327.**
Articles for notation

Neoplastic diseases


- **Purpose:** To evaluate the diagnostic use of cytology fluid tumor markers (squamous cell carcinoma antigen, cytokeratin-19, and CEA) for NSCLC subtyping, and correlate these markers with FDG uptake

- **Methods:** 261 patients underwent transthoracic needle aspiration biopsy (NAB) for a nodule or mass; total of 221 met inclusion criteria (>20 yrs old, solid lesion >8mm with less than 50% GGO)
  - 148 were malignant, 56 benign, 17 indeterminate; only those with pathologically confirmed adca or SqCC were analyzed
  - Blood and cytology fluid (CF) were obtained, then tumor marker assays were performed for CYFRA 21-1, CEA, and SCCA.
  - CF specimen also Pap stained, and histologic classification performed per IASLC/ATS/ERS classification
  - Patients also underwent PET scan.

- **Results:** 88 patients had either adca (n = 58) or SCC (n=30);
  - Concentrations of CYFRA 21-1 were significantly higher in CF samples than in serum
  - CEA levels higher in serum than in CF but not statistically significant
    - Higher CEA levels correlated with distant metastases
  - SCCA significantly higher in CF than in serum
  - In univariate analysis, CF SCCA, serum SCCA, serum CEA, SUV, smoking status, and sex predicted for SCC over adca

- **Take home points:** Of the markers tested, CF SCCA was the most accurate in predicting histology--in practice this may or may not be useful. It might come in handy if histologic or cytologic findings are inconclusive.


- **Purpose:** To study the rates of ALK translocations in patients with advanced NSCLC in a European cancer center, and correlate with rates of EGFR mutations, KRAS mutations, and MET amplification.

- **Methods:** Studied 86 patients with "advanced" (no definition given) NSCLC seen at a single institution--performed ALK FISH, MET FISH in all ALK-positive cases, EGFR and KRAS mutation analysis by PCR followed by sequencing. If ALK-positive, then cases were analyzed for ALK mutations.

- **Results:** Nine (10.5%) tumors were ALK-positive--4 of which were "typical" and 5 of which were "atypical".
  - EFGR, KRAS, MET, and ALK translocations were all mutually exclusive
  - 10 (14.5%) had EGFR mutations
  - 11 (20.8%) had KRAS mutations
No MET amplifications or ALK mutations in the ALK translocation-positive cases.

**Take home points:** The incidence of ALK-translocation tumors in this population was similar to previously reported EGFR wild type populations. The authors also advocated both judicious use of IHC in subtyping NSCLC and performing molecular tests simultaneously in order to conserve tissue in small biopsies.


**Purpose:** To validate an immunohistochemical marker for ERCC1 (mouse monoclonal antibody 8F1) as a predictive marker of response to platinum-based chemotherapy regimens.

**Methods:** Tumor samples from IALT, Cancer and Leukemia Group B 9633, and National Cancer Institute of Canada Clinical Trials Group JBR.10 were included in a biomarker project
  - IHC against ERCC1 performed and interpreted by an "experienced pathologist" in a blinded manor
  - A subset of 150 random samples were "re-analyzed" and interobserver agreement was >95%.
  - Staining graded on a scale from 0 to 3

**Results:** ERCC1 was positive (score of >1) in 78% of samples (n=494)
  - In ERCC1 negative tumors, overall survival did not differ between chemo and no chemo groups
  - In ERCC1 positive tumors, there was some benefit from chemo but this did not reach statistical significance.
  - Interesting that this same antibody was used in this same cohort once before, and only 44% of tumors were positive--the group did not have any of the "old" antibody to figure out why.
  - 14 different commercially available antibodies were tested, and none of them were specific to one isoform of ERCC1, and each recognized at least three different isoforms

**Take home points:** ERCC1 does not seem to be an efficient predictor of the benefit of cisplatin-based chemotherapy in NSCLC patients. The antibody performed very differently now than it did when the same group tested the same tumors 5 years ago, for unknown reasons; therefore, it is important for guidelines to communicate what precisely is tested in immunohistochemical biomarker studies. Currently available antibodies do not have adequate discrimination for therapeutic decision making with regards to platinum-based chemotherapy in these patients.


**Purpose:** To describe the effect of the transcription factor BRN2 on the expression of neural and neuroendocrine-related gene expression in small cell carcinomas of the lung, and to see if it can influence biologic behavior.

**Methods:** Seven SCLC cell line cultures, 6 NSCLC cell lines, and a kidney cell line tested for BRN2 and neuroendocrine related genes (ASCL1, ND1, NCAM1, SYP, chromogranin,
and beta-actin) using RT-PCR and Western blot analysis. Also used retroviruses to transfect cell lines with BRN2, followed by immunocytochemistry.

- **Results:** All SCLC and no NSCLC lines expressed BRN2 and the neural/neuroendocrine markers.
  - When transfected with BRN2, these cell lines had the neural/neuroendocrine markers upregulated.
- **Take home points:** BRN2 is specifically expressed in SCLC, and is involved in the expression of a proneural cell-specific transcription factor and three neuroendocrine marker molecules. BRN2 also has the ability to accelerate cell growth; it therefore may be a therapeutic target in the future.

Kadota K et al. "Thyroid transcription factor-1 expression is an independent predictor of recurrence and correlates with the IASLC/ATS/ERS histologic classification in patients with stage I lung adenocarcinoma." *Cancer* 119: 931-938.

- **Purpose:** To determine whether TTF-1 expression correlates with IASLC/ATS/ERS classification and whether it stratifies patients with respect to disease recurrence.
- **Methods:** Retrospective review of all patients diagnosed with stage I lung adenocarcinoma who underwent surgical resection (n=514; 471 available to construct TMA)
  - Slides reviewed by two pathologists, and classified into AIS/MIA, and invasive adca (further subclassified into lepidic-, acinar-, papillary-, micropapillary-, solid-, and colloid-predominant or invasive mucinous adca...invasive mucinous FURTHER subclassified into pure mucinous or mixed mucinous/nonmucinous--YIKES)
  - Graded by architecture: low = AIS, MIA, lepidic; intermediate=papillary or acinar; high=micropapillary, solid, colloid, or invasive mucinous--not sure if there is data to back up this grading system??
    - Nuclear atypia, mitotic count, pleural invasion, LVI, and necrosis also graded
  - TMA stained with TTF1, intensity was scored
- **Results:** On univariate analysis, sex, sublobar resection, higher stage, higher architectural grade, LVI, necrosis, nuclear atypia, and higher mitotic count associated with disease recurrence.
  - TTF-1 was positive in most tumors, and was most common in low grade but still seen in 80% of high grade tumors; negative TTF correlated with higher mitotic count, larger tumor size, and necrosis
  - Negativity for TTF had a statistically significant increased risk of disease recurrence.
- **Take home points:** Lack of TTF expression is more common in higher grade tumors and is an independent predictor of disease recurrence. Mucinous tumors have less TTF expression


- **Purpose:** To examine the utility of GLUT-1 and IMP3 staining in separating benign from malignant mesothelial proliferations.
- **Methods:** TMA constructed from 78 cases--only cases with "typical histologic patterns" of benign versus malignant were used in order to "make it accurate."
  - 48 benign (27 pleural and 21 peritoneal) mesothelial proliferations and 30 malignant (26 pleural, 3 peritoneal, 1 pericardial) mesotheliomas used
o Stained for IMP3 (insulin-like growth factor II mRNA binding protein 3) and GLUT-1 (glucose transporter protein 1), which were then scored.

- **Results:**
  o IMP3 was positive in 16/30 (53%) of malignant and 13/48 (27%) of benign mesothelial processes, which is statistically significant (sensitivity = 0.53; specificity = 0.73; PPV = 0.55; NPV = 0.71)
  o GLUT-1 was positive in 6/48 (13%) of benign and 18/30 (60%) of malignant cases, which is statistically significant (sensitivity = 0.60; specificity = 0.88; PPV = 0.75; NPV = 0.78)
  o Combined: 13/30 (43%) malignant cases positive for both compared to 2/48 (4%) of benign cases (Table 1; Tables 2 and 3 break down statistics by morphology)

- **Take home points:** GLUT-1 and IMP3 are not helpful on a case-by-case basis as they are not specific. And of course, these markers were applied to cases that were "typical" of benign or malignant mesothelial processes (is there such a thing???) so how will this stain act in cases that are difficult on histology alone?


- **Purpose:** To evaluate the prognostic factors in N1-stage II NSCLC, with emphasis on the significance of different subgroups of N1 lymphadenopathy.
- **Methods:** Retrospective review of 210 consecutive patients who underwent resection for pathologic N1-stage II NSCLC (T1a-2bN1M0) with curative intent--only analyzed patients with >12 regional lymph nodes resected to ensure adequate sampling (n=163)
  o Pre-op workup included physical exam, serum biochemistry, bronchoscopy, chest/abdomen CT, bone scan, CT or MRI of brain, and PET after 2007
  o Node zones defined according to IASLC definition;
  o Outcome defined as death to either cancer or noncancer causes
- **Results:** Total of 163 patients, median follow up time 37.2 months; at last follow up, 62 (37.4%) alive.
  o 1-, 3-, and 5-yr overall survival rates were 85.3%, 62.1%, and 43.5%
  o 90 (55.2%) were adca
  o Univariate analysis found age, cancer-involved lymph node ratio, and cancer involvement of hilar/interlobar lymph nodes had significant influence on overall survival.
  o Hazard of death ratio greater in tumor size >3 cm, poorly-differentiated tumors, and multiple positive N1 lymph nodes--these did not reach statistical significance
  o In multivariate analysis, only histologic grade and positive hilar/interlobar lymph nodes were significant prognostic factors.
- **Take home points:** The authors point out that N1 disease is probably a heterogeneous group with multiple lymph node related factors (direct invasion versus tumor metastasis? solitary versus multiple? hilar versus interlobar?) Lymph node ratio is a significant prognostic factor--though I did not find an adequate description in the paper of how "one" node was defined (fragment taken by surgeon? submitted by surgeon as one node?). The higher the node, the worse the prognosis. The authors also make a claim that tumor grade impacts prognosis, but do not provide any type of definition or objective description of their grading scheme.

- **Purpose:** To describe the histologic, immunohistochemical, and ultrastructural features of a series of 23 signet-ring cell mesotheliomas as compared to signet-ring cell adenocarcinomas of the lung.

- **Methods:** 23 cases of epithelioid mesothelioma with signet-ring cell features (defined as >10% of tumor having signet-ring cell morphology) compared to 7 signet-ring cell adenocarcinomas of the lung.
  - Mucicarmine stain done in "selected" cases
  - EM done on 12 mesothelioma cases and 1 lung adca case.

- **Results:** Two of the meso cases originally diagnosed as adca
  - On H&E, the mesos had areas in which it extensively composed of signet-ring cells, with a large, intracytoplasmic vacuole which displaced nucleus toward periphery of the cell
  - Some meso vacuoles contained bluish stuff, most likely proteoglycans as all were mucicarmine negative
  - IHC: all mesos positive for calretinin (also all positive for CK 5/6, CK 7, and mesothelin); most (9%) positive for WT1 and podoplanin. All negative for MOC-31, CEA, TAG-72, CD15, TTF-1, napsin A, CK20, and CDX2.
  - IHC adcas: all adca positive for CK7, MOC-31, CEA, and napsin; most (6/7) positive for TTF-1; All negative for CK 5/6 CK 20, calretinin, WT1, podoplanin, CDX2
  - EM: The holes in the mesos were intracytoplasmic lumens; those in the adcas were usually due to mucin granules.
• **Take home points:** Signet-ring cell morphology is not uncommon in mesotheliomas--use IHC to figure it out.


• **Purpose:** To see the cost-benefit and cost-effectiveness analysis of EBUS versus CT-PNB for the evaluation of peripheral lung nodules.

• **Methods:** Very complicated computer methods, which involved creating a hypothetical "model" population that could hypothetically be referred to a health care center for a hypothetical biopsy. The only actual data used were healthcare dollars.

• **Results:** CT-PNB was more initially cost-beneficial by $24 compared to EBUS; this remained more cost-effective as time passed. But this depends on a lot of clinical and radiographic factors that are probably institution, clinician, and patient-preference related.

• **Take home points:** Biopsies done by EBUS or CT-guidance are probably equivalent in terms cost-comparisons.


• **Purpose:** Review of primary pulmonary lymphomas.

• **Take home message:** Excellent review of the clinical, radiographic, and histologic features of pulmonary lymphomas, including but not limited to marginal zone/MALT lymphomas, LYG, DLBCL, plasmacytoma, posttransplant lymphoproliferative disorders, ALCL, and Hodgkin. Some (not a ton) of high-quality photos, and also a brief discussion of pleural involvement of lymphomas. A good read if you are looking for a concise source of info on lung lymphoma.

*Non-neoplastic diseases*


• **Purpose:** To evaluate the correlation of capillary inflammation, DAD, and/or complement deposition with lung transplant biopsies from patients with donor specific antibodies (DSAs), non-DSAs, and no identifiable antibodies, and correlate these findings with graft survival.

• **Methods:** Biopsies reviewed retrospectively and grouped based on the presence or absence of a DSA within 28 days of biopsy
  - HLA class I and II typing was performed at months 1, 3, 6, 12 posttransplant, bi-annually thereafter, and at times of suspected rejection.
  - Biopsy slides reviewed for presence/absence of acute cellular rejection (ACR), lymphocytic bronchiolitis (per ISHLT definitions), DAD, constrictive bronchiolitis, and grading of capillary neutrophilia.
  - C4d and C3d staining performed, examined for pattern and intensity of staining.

• **Results:** 41 patients included (16 DSA positive and 25 DSA negative controls--9 of which developed anti-HLA antibodies that were not donor specific)
  - 13/41 diagnosed with ACR (6=A1, 5=A2, 2=A3) and 14/41 with bronchiolitis
26/41 had "greater than baseline neutrophils" in capillaries--more common in DSA than controls
17/41 considered "suspicious for AMR" defined as 2+ capillary neutrophilia and/or DAD--more common in DSA than controls
No correlation with C4d or C3d staining with DSA
DSA plus histology suspicious for AMR correlated with developing BOS or mortality

- **Take home points:** Capillary neutrophilic inflammation (2+ neutrophilia or at least 2 back-to-back neutrophils) and/or DAD positively correlates with DSA. Pathologic findings plus DSA are inversely related to graft survival. C4d and C3d still useless in the lung, according to this group's findings anyway.


- **Purpose:** To describe the pathologic features of the lung cysts seen in BHD patients, so as to make the clinician/pathologist aware of the diagnosis.
- **Methods:** Literature review
- **Results:** Multiple pulmonary cysts and pneumothoraces are frequent in BHD patients (up to 80-100%), which can be confused with LAM, COPD
  - Pathologic features of BHD in the lung include cysts in which the cyst wall expands towards the visceral pleura and is partially incorporated into the parenchyma, interlobular septum, or bronchovascular bundle.
  - Enlarged cysts can be segmented by an alveolar wall, making it look multicystic.
  - Once there is cyst rupture with pneumothorax, these features are often obscured, so the authors suggest that surgeons sample unruptured cysts in cases in which BHD is suspected.
  - There is also a discussion on the symptoms associated with folliculin (FLCN), thought to be the cause of BHD, starting on page 181 if interested.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of the lung cystic lesions between Birt–Hogg–Dubé syndrome (BHD) and blebs/bullae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Lower lobes/perime diastinum</td>
</tr>
<tr>
<td>Number</td>
<td>A few to multiple</td>
</tr>
<tr>
<td>Basic structure</td>
<td>Alveolar cysts/partially fused to pleura</td>
</tr>
<tr>
<td>Lining cells</td>
<td>Often attenuated/ flattened respiratory epithelium</td>
</tr>
<tr>
<td>Stroma</td>
<td>No fibrosis</td>
</tr>
<tr>
<td>Mesothelial invagination</td>
<td>No</td>
</tr>
<tr>
<td>Inflammation</td>
<td>None to mild</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>Not yet</td>
</tr>
</tbody>
</table>

- **Take home points:** In BHD lung cysts, the cyst walls expand towards the visceral pleura, and the inner surface is lined by attenuated alveolar epithelium, as opposed to blebs and bullae which show more fibrosis and do not have cells lining the cysts.

**Purpose:** To describe the pathologic findings (including virus distribution and expression levels of cytokines and chemokines) in post-mortem lung samples taken from patients infected with the H5N1 avian influenza from 2003 through the present.

**Methods:** H5N1 infection confirmed by detecting viral RNA by RT-PCR.
- H&E slides as well as IHC for cell type-specific marker proteins, cytokines, chemokines, and influenza A were performed
- ISH for influenza RNA performed, and double immunofluorescence staining for influenza A, cytokines, chemokines, or cell type-specific marker proteins performed

**Results:** Clinical features of all 5 patients seen in Table 1 on page 359.
- All 5 patients had DAD on H&E
- There were some reactive changes in other organs, but no appreciable inflammatory infiltrates.
- In the lung, most inflammatory cells were neutrophils, monocytes, macrophages with rare CD8-positive T cells
- Viral load was variable from case to case and even within the same lung section of the same case.

**Take home points:** All patients in this series who died of H5N1 influenza clinically presented with ARDS and showed DAD on histology. These patients also had dysregulation of cytokine and chemokine levels (AKA "cytokine storm"). This group was also not able to demonstrate dissemination of H5N1 virus beyond the lung.

Ofek E et al. "Restrictive allograft syndrome post lung transplantation is characterized by pleuroparenchymal fibroelastosis." Mod Pathol 26: 350-356.

**Purpose:** The authors define restrictive allograft syndrome as a distinct form of chronic lung allograft dysfunction demonstrating a restrictive physiology on PFT’s, as opposed to constrictive (obliterative) bronchiolitis, which accounts for 25-35% of chronic lung allograft dysfunction. Here, they report that pleuroparenchymal fibroelastosis is a major histopathologic correlate of this syndrome.

**Methods:** Restrictive allograft syndrome defined as irreversible decline in total lung capacity to <90% of baseline with chronic graft dysfunction; 16 specimens (5 wedge, 4 explants from retransplantation, and 7 autopsies) available.
- Lungs assessed for degree of pleuroparenchymal fibroelastosis, distribution of fibroelastosis, presence of constrictive bronchiolitis, features of uninvolved lung, presence of honeycomb change or other features of a CIP, DAD, and vascular changes

**Results:** All cases showed some degree of pleural fibrosis; 15/16 demonstrated pleuroparenchymal fibroelastosis (defined as confluent areas of bland, hypocellular collagen deposition with preservation and thickening of the underlying alveolar septal elastic network)
Autopsy/retransplant patients more severe than wedge biopsies

The other one specimen had DAD, and the biopsy was taken 2 months before the onset of graft dysfunction

Constrictive bronchiolitis also found in 14/16 cases

DAD present in 13/16 cases

- **Take home points:** Chronic lung allograft dysfunction may have more histologic features than the commonly considered constrictive bronchiolitis--some patients present with restrictive rather than obstructive physiology and have a significantly worse prognosis. The findings of pleuroparenchymal fibroelastosis may correlate with this syndrome--although these patients also had constrictive bronchiolitis in most cases as well.


- **Purpose:** The current gold standard for evaluation lung transplant allograft rejection is through flexible bronchoscopy with Radial Jaw forceps (FTBBx); this group sought to investigate the use of cryoprobe transbronchial biopsy (CPBx) instead.

- **Methods:** 17 consecutive patients undergoing bronchoscopy for allograft rejection evaluation were evaluated. They each first underwent FTBBx with 10 samples taken, followed by evaluation for pneumothorax, followed by 5 CPBx samples taken. These were evaluated by a lung pathologist for total sample area, alveolated area, percent open alveoli, percent artifact-free lung parenchyma, presence of crush/freezing artifact, and presence of rejection graded as A or B.

- **Results:**
  - No statistically significant rates of bleeding between the techniques; no immediate pneumothoraces in either group (one delayed pneumothorax)
Mean sample size, percent open alveoli, rate of crush artifact all statistically significantly improved with the CPBx technique, and no freezing artifact seen

- **Take home points:** CPBx is safe and provides larger, more "artifact-free" specimens than traditional FTBBx in lung transplant patients.