I. Articles for Discussion


**Background:** Establishing a diagnosis in lung cancer requires histologic or cytologic specimens (biopsies), which are invasive, time-consuming, associated with morbidity, and are often non-diagnostic (sampling error). Multiphoton microscopy (MPM) offers the promise of real-time in-situ visualization of all suspicious lesions at the cellular and subcellular level, with biopsy and resection of only pathologic lesions, reducing sampling error and morbidity.

MPM – Can be used in-vivo or ex-vivo to generate images at histologic resolution of fresh, unprocessed, unstained tissue using absorption of 2-3 low energy (near-infrared) photons to cause non-linear excitation to generate intrinsic tissue emission signals (and lots of other complicated physics which are detailed in the supplement and other references).

**Purpose:** Proof-of-principle pilot study exploring the use of MPM as a new optical biopsy tool for the detection and diagnosis of lung tumors in real time.

**Methods:** A section of tumor and non-tumor were taken from 25 lobectomies for lung cancer. Images were acquired using the MPM imaging system at X4 (0.5 mm depth) and X25 (0.25 mm depth). Each image takes about 1-3 seconds to generate. After MPM imaging the specimens were processed for histology.

Blinded diagnostic analysis – a pulmonary pathologist and a general surgical pathologist were trained in MPM images, and their diagnoses on MPM were compared with the same specimen on histology.

**Results:**

Let’s look at some of the images…
Elastin = color-coded green (short wavelength)

Collagen = color-coded red

Smoker’s macrophages = color-coded blue (long wavelength)

Lymphocytes = color-coded green
Spectrum of atypical glandular lesions, from AAH to well-differentiated lepidic-predominant adenocarcinoma to invasive solid adenocarcinoma to papillary adenocarcinomas

Other images (not copied here but in the paper) – show correlation between amount of collagen and degree of differentiation in adenocarcinoma.
A-B: Correctly diagnosed as squamous cell carcinoma.

C-D: Squamous cell carcinoma incorrectly diagnosed as adenocarcinoma.
“Adequacy assessment” – specimens lacking viable (or any) tumor, correctly assessed by MPM.

How did the pathologists do?

23 (of 25) samples with diagnostic material.

15 of 16 (94%) adenoca, 4 of 7 (57%) squamous cell
Comment:

1. MPM joins a growing array of non-invasive real-time optical biopsy techniques (optical coherence tomography, confocal endomicroscopy, endocytoscopy). These other techniques apparently cannot generate the level of histologic detail, or penetrate the tissue as deeply, or require contrast agents (i.e. – MPM most similar to classic histopathology).

2. The diagnostic potential of MPM imaging has been explored in multiple prior studies in multiple organ systems in both animal and human models to differentiated normal from diseased tissue and benign for malignant conditions, but the authors claim that this is the first study assessing its potential to generate specific “histologic” diagnoses.

3. Miniaturized MPM devices already available and under investigation (bronchoscopy, laparoscopy, interventional radiology)

3. MPM shows potential in (1) increasing yield of diagnosable tissue, (2) minimizing unnecessary biopsies, (3) assessing surgical margins, and (4) as a diagnostic modality (??).


Caveat – This is not a pathology study. I choose to include it as a springboard for discussion about this diagnosis.

Background/ methods: Pleuroparenchymal fibroelastosis (PPFE) has been described as an uncommon form of idiopathic interstitial pneumonia, and as a form of chronic GVHD of the lung in allogeneic BMT patients and chronic rejection in lung allografts.

This report is a case series of 6 patients alleged to have developed “PPFE” as a consequence of prior chemotherapy. The patients presented with “bi-apical pleural fibrosis encroaching the lung, with a clinical, radiographic, and physiological presentation [details not provided] suggestive of PPFE.”

Onset of symptoms – insidious with slowly progressive dyspnea, non-productive cough, and pleuritis chest pain.

Chest radiographs – normal prior to therapy.

Four patients had the diagnosis of “PPFE” confirmed on surgical biopsy of lung and pleura [not provided]. In the other 2 patients, a biopsy was deemed not necessary due to “typical” imaging findings and attendant risk.
Clinical course: Patients 2-5 died. Patients 1 and 6 showed deterioration.

Comments:

1. Another report that “muddies the water” on PPFE as a pathologic entity.

2. These patients must have all had diffuse lung disease (undoubtedly drug-induced), just not sure it should be called “PPFE”.

4. Most of the “pathologically proven” PPFE cases in the literature (or in manuscripts) that I have come across has been indistinguishable from (a) simple apical cap OR (b) apical cap + another well-defined chronic interstitial pneumonia (such as UIP or NSIP), OR (c) upper-lobe involvement in fibroelastotic-rich UIP.

4. How is PPFE to be distinguished from apical cap?
5. The only times I have come across PPFE is in the settings of uncommon upper lobe fibrosis in (a) chronic rejection (restrictive allograft syndrome/ PPFE) and (b) GVHD of the lung in allo-BMT patients (PPFE). The fibrosis looked NOTHING LIKE an apical cap, but bore some resemblance to UIP.


Background: ROS1 is an integral membrane protein and a member of a tyrosine kinase insulin receptor subfamily with tyrosine kinase activity. ROS1-rearranged tumors may be druggable targets (crizotinib, pemetrexed). About 0.85 – 1.7% of NSCLCs (almost all adenoca) have been shown to have ROS1 rearrangements as evaluated by FISH or sequencing-based approaches. Prior reports have shown correlation between confirmed ROS1 translocations and ROS1 IHC. This report is undertaken to provide (a) broader ROS1 prevalence data, (b) correlate IHC and FISH analyses, and (c) correlate with clinicopathologic characteristics.

Methods: FFPE from 1478 completely resected NSCLC from 2002-10 from a German hospital:

- 47% adenoca, 39.9% squamous cell, 4.8% large cell, 3.5% adenosquamous, 2.7% pleomorphic carcinoma, 1.4% large cell neuroendocrine, and 0.7% “mixed morphology”.
- UICC stage I (36.2%), stage II (30.2%), stage III (31.1%), stage IV (2.5%)
- Only 3% treated with less than lobectomy (wedge or segmentectomy)
- 25.5% adjuvant chemotherapy, 15.6% adjuvant radiotherapy
- For 405 adenoca, data also available on EGFR, KRAS, BRAF, and ALK.
- ROS1-specific Ab (clone D4D6): cases grouped as either negative (0) or positive (1+ to 3+) cytoplasmic staining
- FISH (dual color break apart probe) done all IHC-positive cases (>15% tumor cells with split signals).

Results: 68 total cases were ROS1 IHC positive:

- 8.9% of adenoca ROS1 IHC + (1+ = 30, 2+ = 20, 3+ = 11) \( \rightarrow \) comprised 61 cases
- 0.5% of squamous cell (1+ = 2, 2+ = 1); 1.9% of adenosquamous (1+ = 1)
- 2.8% of large cell (2+ = 2), 2.5% of pleomorphic (3+ = 1)
Of those 68 cases, only 9 showed split signal by FISH:
- 2 had 1+ IHC score// 2 had 2+ IHC score// 5 had 3+ IHC score
- 8 adenoca, 1 large cell

Clinicopathologic correlates:
- ROS1 translocation (and IHC) more common in woman than men
- ROS1 IHC positive correlates with low pT stage
- ROS1 IHC/ translocation corresponds with adenoca histology (lepidic, acinar, solid)
- TTF-1+ seen in 44 of 47 (93%) of IHC+ and 100% (presumably 9 of 9) ROS1-translocated
- Of 6 ALK-translocated adenoca, none had concomitant ROS1 translocation
- Of 64 tumors with EGFR mutation, 1 had concomitant ROS1 translocation

Their take-home messages: - Adenoca histology, female sex, low pT stage, TTF-1 expression: enriched for ROS1 translocation
- ROS1 IHC expression is stage-independent predictor of improved OS
- Screening by ROS1 IHC will reliably identify pts with ROS1 translocations
- Crizotinib inhibits ROS1 protein and not ROS1 rearrangements, so maybe ROS1 expression more relevant (perhaps suggesting tumor-dependent signaling in absence of translocation)
My take home message:

- ROS1 IHC not useful in evaluating for ROS1 translocation.


**Background:** Beginning in February 2011, a cluster of young, previously healthy adults were admitted to the ICU in a hospital in Seoul, S. Korea with severe respiratory distress for which no etiology could be discovered. The cases were uniformly refractory to therapy (incl. antivirals and immunosuppression), and progressed to death or lung transplantation. Several other patients in other parts of the country were identified with similar features, and infant cases and familial clustering was discovered. Initially, a novel viral epidemic was suspected but was ruled out after extensive microbiological investigations.

**Methods:** Multidisciplinary conferences were chaired and organized, and 3 aspects became recognizable: (1) Radiologic and pathologic findings suggested inhalational injury, (2) occurrence in winter to early spring, and (3) many of the patients were peripartum women, who were staying indoors. This raised suspicion for possible inhalational toxins in the home, including humidifier disinfectants. A case-control epidemiological study was undertaken with collaboration with the Korean Centers for Disease Control (KCDC).

**Results:** 17 cases of humidifier disinfectant-associated lung injury (HDALI):

- 5 died, 4 underwent lung transplantation (and survived)
- 6 were pregnant at presentation, 4 had given birth 2 weeks earlier
- Cases peaked in April, ended by June
- Clinical features: dyspnea, cough, fever (20%); 10 of 13 admitted pts developed ARDS
  
  ICU mortality rate = 50% (5 of 10 pts)

  PFTs (9 patients): restrictive physiology

- Radiologic features

  Early stage: Multifocal, patchy consolidation, lower lung zones, subpleural sparing

  Later stage: Diffuse centrilobular ground glass opacities
Pathologic findings: 14 specimens obtained from 11 patients - 

-- 4 – explanted lungs, 3 – autopsy lungs, 5 – wedge resections, 1 – TBBx, 1- CNBx

-- All showed “bronchiolocentric, temporally homogenous, acute lung injury pattern”

7 specimens taken during “early stage” –

“Uneven bronchiolar wall thickening with subepithelial fibroblastic proliferation”, felt to be consistent with obliterative bronchiolitis

“Alveolar septa showed septal expansion due to lymphoplasmacytic infiltration and a hyaline membrane [sic] accompanied by alveolar pneumocytes hyperplasia. Intralveolar fibroblastic plugs and intra-alveolar macrophages were observed frequently.”

7 specimens taken during the “later stage” –
“Bronchiolar destruction with scarring…alveoli were remodeled by inflammation and fibrosis. Interstitial fibroblastic proliferation and intra-alveolar fibroblastic plugs with mural incorporation were observed. Residual hyaline membrane was identified in some cases. Even though four of the seven late-stage specimens showed end-stage lung fibrosis, peripheral lobular air space preservation and obliterative bronchiolitis pattern were maintained. None of the cases exhibited granulomatus lesions or old mature fibrosis, including smooth muscle metaplasia and microscopic honeycomb change.”

(According to them) Taken separately, the lesions overlapped with DAD, hypersensitivity pneumonia, BOOP, acute fibrinous and organizing pneumonia (AFOP), and obliterative bronchiolitis. Collectively, they “constitute a distinctive lung injury entity.”

Subsequent events – humidifier disinfectants withdrawn from the market and no cases reported in 2013 and 2013.
Comments:

1. Very interesting report of rapidly progressive severe acute lung injury due to humidifier disinfectant.

2. Limitations – unable to comment on the prevalence of less severe disease due to humidifier disinfectant.

3. The pathological descriptions are a little sketchy – I suspect much of what they describe is mostly just the acute and organizing phases of DAD. I remain a little skeptical that obliteratorive bronchiolitis can develop over such as short (2-3 weeks) time course (and patients did not show obstructive physiology).