PULMONARY PATHOLOGY JOURNAL CLUB
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I. Articles for Discussion


**Background:**
- In difficult cases, IHC may be used to diagnose LAM and HMB45 can be patchy and difficult to interpret.
- Aberrant β-Catenin signaling has a potential role in the pathogenesis of LAM and β-Catenin shown to be positive in cases of sporadic LAM.

**Aim:**
- To look at the role of β-Catenin in the diagnosis of LAM.

**Methods:**
- IHC for β-Catenin in 28 cases of LAM, 10 cases of renal angiomyolipomas.
- Controls included 6 cases of chronic bronchitis/emphysema, 5 PLCH, and the normal lung adjacent to lesional tissue.
- Compared IHC of β-Catenin to that of ER/PR and HMB45

**Results:**
- 100% staining of LAM with β-Catenin, mod to strong versus 100% HMB45 staining mild-moderate. ER was 71%
- 80% staining of angiolipomas with β-Catenin
- All the controls were negative

**Take home message:**
- β-Catenin a good stain, very specific. What they didn’t mention is that leiomyomas are negative for β-Catenin so in that differential also helpful.


**Background:**
- EGFR mutation occurs in adenocarcinoma (AD) of the lung but not squamous cell carcinoma (SQCC).
- No study looking at EGFR and k-ras mutations in adenosquamous cell carcinomas (ADSQCC) in non-Asian population

**Purpose:**
- To assess for EGFR and k-ras mutations in ADSQCC in non-Asian population and review the literature on Asian population.

**Methods:**
- 23 cases of well-diff or mod diff ADSQCC
- Microdissection of the adeno and squamous component separately
- EGFR and k-ras mutations assessed in each component
- FISH for EGFR assessed in each component.

**Results:**
- 3/ 23 cases had EGFR mutation and 3 /23 k-ras mutation, mutually exclusive.
- For both EGFR and k-ras, 2/3 had the same mutation in both glandular and squamous component but 1/3 had a difference between squamous and glandular components.
EGFR amplification was noted in 11 cases in both components. All cases mutated were amplified but not all amplified cases were mutated.

**Take Home Message:**
- EGFR mutation in Western population, AD about 10% and in this study in ADSQCC 13% so similar. In Asian population, ADSQCC similar at 40% in Korean and dissimilar in Japanese with less mutations in ADSQCC at 15% (difference in methodology)
- All mutations in women both Asian and Western population
- K-ras mutations more in ADSQCC than SQCC but less than AD.
- **So we should look for EGFR and k-ras mutations in ADSQCC like we do in AD.** The question I can’t find the answer for is should we always do both components separately? Their data would suggest best to do it that way.


**Background:**
- The prognosis and therapeutic decision in MPM is currently based on histologic subtype and TNM staging.
- Epithelial MPM have the nest prognosis but they are histologically heterogeneous and no studies have been done to look at the clinical significance of these histologic subtypes (analogy to lung adenocarcinomas).

**Aim:** To correlate the histologic subtype of epithelial MPM with clinical features and investigate the biologic significance.

**Method:**
- Study population of 232 epithelial, 47 biphasic and 26 sarcomatoid MPM.
- Histologic features assessed in increments of 5%: trabecular, tubulopapillary, micropapillary, solid and pleomorphic.
  - Classified as pleomorphic is more than 10% of tumor like pleomorphic ca of lung (anaplasia, giant cells).
  - Other MPM classified according to predominant subtype.
- Also assessed “cytologic” features and called + if present in >10% of tumor: adenomatoid, clear cell, deciduoid and small cell
- Stromal features called + if myxoid >50%
- Lymphovascular invasion assessed

**Results:**

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Clinicopathologic Factors by Five Histologic Subtypes in 232 Patients with Epithelioid DMPM</th>
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<tbody>
<tr>
<td>No.</td>
<td>All Patients</td>
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<td></td>
<td>Percentage</td>
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</tbody>
</table>
Pleomorphic subtype independent prognostic factor in multivariate and in OS falls with biphasic and sarcomatoid.

Take home message

- Pleomorphic MPM to be re-classified with sarcomatoid MPM

4- Yoshizawa et al (Travis as senior author) Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. Mod Pathol 2011; 24: 654-44

Background:
- The New Proposed Classification of Lung Adenocarcinoma.

Aim: To explore to prognostic significance of this classification in a large series of surgically resected Stage I adenocarcinomas.

Method:
- Previously untreated Stage I according to the 7th TNM edition, adenocarcinomas resected at MSK.
  - All H&E slides reviewed by 1 pathologist and “problem” cases reviewed by 2 others including WDT. Average # slides per case 8.6
  - Used the new proposed classification and recorded subtypes in 5% increment. And the predominant pattern defined as the pattern with the largest %.
  - Size measured as total tumor size and invasive component not included into lepidic growth
    - For invasion measured 2 ways, if area on one slide, with ruler. If larger and not measurable on single slide, total size X % non lepidic growth
- Looked at pleural invasion as PL0, PL1 and PL2
- Assessed tumor grade, vascular invasion and necrosis
- OS and DFS

IASLC/ATS/ERS | Number (%) | 2004 WHO | Number (%) |
--- | --- | --- | --- |
NA | Mixed subtype (MS) | 490 (95) |  |
Adenocarcinoma in situ | 1 (0.2) | BAC, non-mucinous | 1 (0.2) |
Minimally invasive adenocarcinoma, non-mucinous | 7 (1.4) | NA (included in mixed subtype) | 0 |
MIA, mixed mucinous and non-mucinous | 1 (0.2) | NA (included in mixed subtype) | 0 |
Lepidic predominant | 29 (5.6) | NA (included in mixed subtype) | 0 |
Acinar predominant | 232 (45.1) | NA (included in mixed subtype) | 0 |
Papillary predominant | 143 (27.8) | Acinar | 6 (1.2) |
Micropapillary predominant | 12 (2.3) | Papillary | 11 (2.1) |
Solid predominant | 67 (13) | NA | 0 |
Variants Invasive mucinous adenocarcinoma (and mixed mucinous/non-mucinous) | 0 | Colloid | 4 (0.8) |
Colloid predominant NA | | Signet ring | 1 (0.2) |

Histological subtype | N (%) | Mean age yrs (range) | Sex (M/F) | Race W/O | Smoking C/F/N (MPY) | Stage IA/B | Mean gross size cm (range) | Mean invasive size cm (range) |
--- | --- | --- | --- | --- | --- | --- | --- | --- |
AIS or MIA | 9 | 68 (52–81) | 4/5 | 6/3 | 0/8/1 (23) | 9/0 | 1.3 (0.5–2) | 0.2 (0–0.3) |
Lepidic predominant | 29 (5.6) | 69 (43–84) | 17/12 | 28/1 | 1/22/5 (19) | 17/12 | 2.3 (0.9–4.6) | 1 (0.3–2.8) |
Acinar predominant | 232 (45.1) | 68 (33–89) | 71/161 | 209/23 | 29/158/45 (36) | 138/94 | 2.1 (0.3–5.0) | 1.9 (0.3–5.0) |
Papillary predominant | 143 (27.8) | 67 (42–87) | 55/88 | 130/13 | 28/140/18 (46) | 88/55 | 2.2 (0.7–5.0) | 1.9 (0.6–5.0) |
Micropapillary predominant | 12 (2.3) | 72 (61–86) | 6/6 | 12/0 | 7/3/3 (38) | 6/6 | 2.8 (1.5–5.0) | 2.8 (1.5–5.0) |
Solid predominant | 67 (13) | 66 (43–83) | 29/38 | 62/5 | 15/50/1 (59) | 28/39 | 2.5 (0.5–5.0) | 2.5 (0.5–5.0) |
Invasive mucinous and mixed mucinous/non-mucinous | 13 (2.5) | 71 (54–85) | 4/9 | 13/0 | 5/5/3 (37) | 7/6 | 2.5 (0.5–3.6) | 1.1 (0.3–1.8) |
Adenocarcinoma Colloid predominant | 9 (1.8) | 62 (49–77) | 5/4 | 9/0 | 3/4/2 (38) | 5/4 | 2.5 (1.3–5.0) | 2.3 (1.0–5.0) |
Total | 514 | 68 (33–89) | 191 (37%)/323 (63%) | 469 (91%)/45 (9%) | 75 (15%)/358 (70%)/78 (15%)/41 | 298 (58%)/216 (42%) | 2.2 (0.3–5.0) | 1.9 (0.3–5.0) |

Abbreviations: AIS, adenocarcinoma in situ; ATS, American Thoracic Society; C, current; ERS, European Respiratory Society; F, former; IASLC, International Association for the Study of Lung Cancer; MIA, minimally invasive adenocarcinoma; MPY, mean pack years; N, never;
Results:
- The results are in tables above.
- Based on survival 3 groups emerged

<table>
<thead>
<tr>
<th>Low grade</th>
<th>DFS 5 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIS</td>
<td>1</td>
</tr>
<tr>
<td>MIA non mucinous</td>
<td>7</td>
</tr>
<tr>
<td>MIA non and mucinous</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intermediate grade</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Lepidic</td>
<td>29</td>
</tr>
<tr>
<td>Acinar</td>
<td>232</td>
</tr>
<tr>
<td>Papillary</td>
<td>143</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High grade</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Micropapillary</td>
<td>12</td>
</tr>
<tr>
<td>Solid</td>
<td>67</td>
</tr>
<tr>
<td>Invasive mucinous</td>
<td>13</td>
</tr>
</tbody>
</table>

- In multivariate, the strongest predictors for DFS were Gender (HR of 1.79, p = 0.007), Necrosis (HR2.15 p=0.002), Tumor size (adjusted for lepidic growth) (HR 1.29 p=0.026) but then Stage IA vs IB didn’t matter with p =0.19, Tumor “grade” ie the 3 groups above (HR 1.7 p=0.038).

Take home message:
- Why split so much if in the end comes down to dividing things in 3 significant groups.
- Based on small number of cases – surprising that the Stage didn’t matter in their multivariate and not clear if this is because of they way they adjusted based on invasion not tumor size?
- Still don’t get the assessment of pleura and having cases in here (or if removed not clear but total numbers remain the same) as they should be stage II?

5- Kaira et al. MUC1 expression in thymic epithelial tumors: MUC1 may be useful marker as differential diagnosis between type B3 thymoma and thymic carcinoma. Virchows Arch 2011; 458: 615-20.

Background:
- Subset of thymomas and thymic carcinomas display aggressive behavior.
- Beyond the WHO classification, identification of biomarkers to predict behavior would be helpful.
- MUC1 plays a role in development and progression of malignant tumors, is a target in immunotherapy for various cancers and has been reported to be associated with prognosis in various malignant tumors.

Aim: Elucidate role of MUC1 in thymic neoplasms.

Methods:
- 55 consecutive patients with thymic epithelial neoplasms with available clinical information.
- Reported as 3 subgroups: 27 low risk thymomas (5A, 17AB, 5B1), 11 high risk thymomas (6B2, 5B3) and 17 thymic carcinomas (8SQCC, 4LCC, 1 basaloid and 2 others).
• IHC for MUC1 (0=0%, 1= <5%, 2= <10%, 3 =10-25%, 4>25% and >10% was called positive), VEGF, CD34 to do MVD, p53

Results:
• MUC 1 scoring illustrated below. 29% of cases had overexpression of MUC1 i.e. score of 2 and more. 0% in low- and high- risk and 94% of thymic carcinomas.

![MUC1 scoring graph](image)

• In univariate, multiple variables statistically correlated with survival including MUC1 but in multivariate only thymoma vs thymic carcinoma was stat significant, not WHO or staging or MUC31 (although surprising since MUC31 seems to correlate almost perfectly between thymomas and thymic carcinomas).
• 0 cases of B3 positive for MUC1 and 94% 16/17 of thymic carcinomas.

Take home message
• As this study, other studies show a difference in survival between thymomas and thymic carcinomas, even B3 and thymic carcinomas.
• The differential diagnosis of B3 vs thymic carcinomas can be challenging and this seems like an excellent marker. Would be nice to try and reproduce.

6- Young et al. Neuroendocrine Cell Distribution and Frequency Distinguish Neuroendocrine Cell Hyperplasia of Infancy From Other Pulmonary Disorders. Chest 2011; 139: 1060-71

Background:
• Neuroendocrine cell hyperplasia of infancy (NEHI) is poorly understood with
  o presentation in first yr of life with tachypnea, retraction, wheezing and no sustained response to bronchodilators or steroids
  o CT scan findings described as very specific but only 78% sensitive with geographic GGO in middle lobes and airtrapping elsewhere.
  o Histologically no formal criteria but suggestions that finding NEC ≥ 70% of bronchioles and ≥ 10% in individual airway c/w dx in appropriate clinical setting.
• However NECH described in many other pediatric diseases like BPD, CF, PHTN
• Furthermore NEHI may be histologically broader than simply having increased NECs

Aim: To study cases of NEHI and compare to other diseases reported to show NECH

Method:
• Study population
  o 13 patients with NEHI based on very stringent clinical and radiologic data.
  o Other diseases selected – age matched and balanced for sample size
    • 3 PHTN
    • 2 BPD/PHTN
    • 2 BPD
• 1 PIG
• 5 Bronchiolitis, acute/chronic/necrotizing/follicular/constriction
  o Control group of normal lung from lobectomy for CCAM
• PFTs
• IHC for bombesin and Ki-67
• Morphometric studies
  o Proximal airways (membranous and respiratory bronchioles) vs respiratory
    (alveolar ducts)
  o For each airway, IHC expressed as % of total epithelial area - % NEC area
  o NE bodies in alveolar ducts divided by the area of tissue sections - % NEB area
  o If more than one biopsy, aggregates of all airways counted

Results:
• The traditional criteria are sensitive but not specific
  o 100% of NEIH had NEC in ≥ 70% of bronchioles and at least one airway with ≥
    10% NECs
  o 5/13 others and 5/6 normal also had NEC in ≥ 70% of bronchioles
  o 50% of others had at least one airway with ≥ 10% NECs
• The total % NEC was the most useful discriminatory. Not only significantly higher but
  also no overlap between values. Although the average values for other metrics higher for
  NEIH, overlap with others and controls.

![Graphs showing NEC area and NEB area](image)

• In patients with NEIH, correlation between the %NEC and FEF at 75% and 85%
• No correlation between histologic findings and imaging

Take home message
• Well done study identifying a specific morphometric abnormality to separate NEIH from
  other conditions resulting in NEC. However, not sure how easily applicable in clinical
  routine.

II. Articles for Notation

Original Articles
1- Fudala et al. Increased Levels of Nuclear Factor kB and Fos-Related Antigen 1 in Lung
Tissues From Patients With Acute Respiratory Distress Syndrome. Arch Pathol Lab Med
2011; 135: 647- 654

Background:
• Proinflammatory signaling initiates epithelial cell dysfunction and apoptosis in
  ARDS/ALI, and nuclear factor kB (NFkB) plays a central role in this process by
  regulating transcription of many inflammatory molecules.
• NFkB interacts with transcription factors from the jun and fos families, including FRA-1
  from the later.
Experimental studies have shown that activation of NFkB can play an important role in ARDS/ALI and FRA-1 activation in lungs of guinea pigs exposed to Mustard gas analog. **Purpose:** To assess lung tissue, normal and from patients with ARDS for NFkB, thought to contribute to dysfunctional inflammation and FRA-1, resulting in cell death.

**Methods:**
- Laser confocal microscopy on 5 tissues from ARDS and 3 normal.
- For each, 1 slide stained with activated NFkB and SurfB and 1 slide with FRA-1 and SurfB.
- Number of epithelial cells + for NFkB or FRA-1 were counted.

**Results:**
- Number of positive epithelial cells for activated NFkB in ARDS is significantly higher than in normal (88.5 vs 3.7; \(p<0.001\)).
- Number of positive epithelial cells for FRA-1 in ARDS is significantly higher than normal (86.2 vs 2.5; \(p<0.001\)).
- Partial co-localization of NFkB and FRA-1 in ARDS.

**Take Home Message:**
- NFkB and FRA-1 possibly play a role in the epithelial cell dysfunction in patients with ARDS.

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2. **Boland et al.** *Pleuropulmonary Infection by Paragonimus westermani in the United States: A Rare Cause of Eosinophilic Pneumonia After Ingestion of Live Crabs.* AJSP 2011; 35:707-13

**Background/Method:** Report of 4 cases with this infection to increase our awareness about the disease.

**Results:**
- 3M, 1F, age 20-66, with variable clinical and radiologic presentation:
  - SOB
  - Non-productive cough
  - Hemoptysis
  - Peripheral eosinophilia
  - Cavitary mass
  - Waxing and waning masses
  - Pleural effusion
  - Pneumothorax
- Histologically, some variability
  - All 4 had CEP with organization
  - All had giant cells
  - 3 with necrotizing granulomas
  - 2 with non necrotizing granulomas
  - 2 had visible eggs
  - Features s/o vasculitis with geographic necrosis, vascular inflammation
- 3 had positive serologies and 2 had history of live crab ingestion history.

**Take Home Message:**
- Another differential of CEP to add to our list and to be aware of.
3- Okamoto et al. Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. ERJ 2011; 37: 1119-27

Background:
- UIP fibrotic lung disease with poor prognosis. COP and cNSIP much better prognosis and fNSIP with intermediate prognosis.
- UIP is associated with overproduction and disorganized deposition of extracellular matrix (ECM) proteins.
- Periostin is and ECM shown to be involved in various pathophysiological statuses of fibrosis, including the healing process in MI and other.

Aim: To assess IPP for periostin and see if correlates with serum levels.

Methods:
- 92 patients, 51 with IPF, 20 with fNSIP, 7 with cNSIP and 14 with COP
  - Diagnosis according to ATS/ERS statement. 28 IPF had SLBx as UIP, all NSIP had SLBx, and COP dx on both SLBx and TTBx
  - Most got serum taken at time of diagnosis
  - Control serum age-matched healthy donors and normal lung tissue non smokers who underwent lung “extirpation”?
  - BALF, PFT
- Created their own antibodies both mono and polyclonal against periostin.
- IHC on paraffin and ELISA on serum

Results:
- IHC study showed periostin to be deposited in fibroblasts/fibrosis of UIP and fNSIP, not in epithelial cells or macrophages. [In fact, very nice and clean immuno.] Was also seen in the fibroblastic plugs of COP. Findings stat significant.

% of cells

<table>
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<tbody>
<tr>
<td>UIP</td>
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<td>fNSIP</td>
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<tr>
<td>cNSIP</td>
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<tr>
<td>COP</td>
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<tr>
<td>control</td>
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- Using serum periostin, IPF patients could be separated from COP and controls.

Take home message
- Very interesting finding in a nicely illustrated and well done study.
- Not practical in that histologically we are not likely to confuse UIP with COP or cNSIP but with fNSIP. Similarly, not likely to have problems clinically and radiologically to separate UIP from COP. But something to build on.

4- Machida et al. Relationship of aquaporin 1, 3, and 5 expression in lung cancer cells to cellular differentiation, invasive growth, and metastasis potential. Human Pathol 2011; 42: 669-678

Background:
Aquaporins (AQP) are channel-forming membrane proteins that play a physiologic role in lung development and fluid transportation, and shown to possibly have an oncogenic role in different tumors with rare reports looking at lung cancer.

Little is known about the preferential expression of AQPs in ADC subtypes and their relation to ADC's prognostically adverse factors.

**Aim:** To look at AQP1, 3 and 5 in various lung cancer subtypes, as well as non-neoplastic lung tissue, elucidate the role of AQP in tumorigenesis, cellular differentiation and invasive growth.

**Methods:**
- Cases included
  - 160 resected lung cancers
    - 125 ADC: 24 Non-mucinous BAC, 27 Mixed adeno-BAC with prominent BAC, 31 mostly invasive well-diff and mod diff adeno, 19 pure mucinous BAC or predominantly BAC, 24 poorly diff adeno. 20 cases had a 30% or more micropapillary component.
    - 18 SQCC
    - 51 LCC
    - 12 SCLC
    - 2 AAH
  - 15 non neoplastic
- IHC on paraffin for AQP 1 and 5, and on frozen for AQP3
- IHC for EMA, Ki-67, p53 and CEA
- Western Blotting for AQP1 on subset of cases
- LCM and qRT-PCR on subset of cases.

**Results:**
- IHC study:
  - AQP1, 3 and 5 were expressed in type II pneumocytes, apical surface of bronchiolar cells
  - AQP1, 3 and 5 in tumor cells 71%, 40%, 56% respectively of cases.
  - AQP1, 3 and 5 higher in well-diff and mod diff than poorly or undiff.
  - AQP1 and 5 neg in SQCC
- Western blot
  - APQ1 overexpressed in lung cancers, except SQCC, versus non-neoplastic, more so in invasive adeno
- qTR-PCR
  - AQP1 and 5 increased in invasive adeno and mucinous adeno
  - AQP3 increased in BAC and decreased in adeno and mucinous adeno
- AQP1 related to post-op metastasis but in multivariate analysis none showed independent prognostication.

**Take home message**
- New interesting molecule to explore in the pathogenesis of lung cancer
- No clinical use at this point.


**Background:**
Endobronchial biopsies are considered gold standard in the assessment of airway inflammation in asthma and often used in research.

But is it truly representative as smaller airways also involved in asthma and do they show same inflammation patterns, as biopsy is superficial limited it the inner airway wall and is distribution of inflammation same in small and large airways?

**Aim:** To assess if mast cell density in large airways related to mast cell density in small airways.

**Methods:**
- Post-mortem whole lungs of 10 subjects dying of non-respiratory cause.
- 6 random biopsies taken from large airways.
- Small airways sampled in transverse sections of lung parenchyma and qualified as small if long to short diameter ratio was 3 or less and perimeter of BM less than 8 mm. Average 11 small airways per case were assessed.
- IHC for tryptase done.
- Cell count using computer and inner wall (WAi) and deep (more than 100 microns) separated from total airway wall (WAit).

**Results:**
- In small airways, mast cell density of WAi similar to large airways but for WAit was 1.4-1.6 fold higher.
- Mast cell density of WAi on bx correlated with WAit in small airways in sections but no correlation between deep on bx and WAit.
- No correlation between WAi in biopsies and WAi in resections.
- 3 biopsies necessary to show variance in mast cell densities.

**Take home message**
- Mass cell density varies between WAi in biopsies and WAit of small airways but is correlated and variance appreciated with 3 biopsies.
- So basically endobronchial biopsies can be good surrogate to assess mast cell counts but does not necessarily apply to other inflammatory cell types. So more experiments for cells like eosinophils and lymphocytes would need to be done.

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**6- Andrews et al. Routinely Obtained Diagnostic Material as a Source of RNA for Personalized Medicine in Lung Cancer Patients. JTO 2011; 8: 884-88.**

**Background:**
- More and more ancillary studies needed on smaller and smaller samples of tissue for lung cancer.
- IHC and DNA extraction even on small samples routinely established. RT-PCR for RNA more problematic.

**Aim:** To define minimum area of tumor tissue required for successful RNA extraction from a range of routinely prepared small samples including cell blocks from thin preps after manual dissection and compare to resected tumors.

**Method:**
- Study conducted in 2 parts
  - 5 lobectomy specimens processed to assess effects of fixation and storage and to work-up assays with respect to tumor area
    - Up to 6 weeks of storage
    - Specimen size tested from 1mm² to >15 mm²
Routinely processed specimens
- 10 biopsies paired to resected specimens
- 10 cytologic cases with associated cell block
Measurement of tumor area by digital imaging analysis on 100 biopsies
RNA extraction and RT-PCR done according to FDA approved, commercially available technology and test used was ResponseDx:Lung

Results:
- The average tumor area on biopsies is 1.34 mm$^2$ and ten 10-micron sections could be achieved.
- As little as 1 mm$^2$ is required for successful RNA extraction.
- Usable RNA was obtained from all routinely processed biopsies and cytologic material using either tumor area > 1 mm$^2$ from a 10 micro section or >800 nuclei as counted on H&E.
- Looking at ResponseDx:Lung, same prediction for ERCC-1 between biopsy and resected tumor and 80% for RRM-1

Take home message
- Good practical study
- Showed can get good quality RNA (although no agilent traces and is actin enough to say good quality) and enough RNA for the specific assay ResponseDx:Lung from biopsy as small as 1 mm$^2$ and cell blocks routinely processed and stored. But no where do they tell us the amount of RNA extracted and assays are different in the amount of RNA that may be needed.

7- Balatti et al. MicroRNAs Dysregulation in Human Malignant Pleural Mesothelioma. JTO 2011; 6: 844-51

Background:
- Asbestos carcinogenesis of MPM linked to nuclear factor-κB activation, Akt activation by phosphorylation
- A3AR (A3 adenosine receptor) reduces Akt phosphorylation and nuclear factor-κB activation in MPM cell lines and decreases proliferation and increases apoptosis of MPM cells. So potential therapeutic target.
- No good treatment for MPM and knowing the molecular pathways of MPM can help in defining novel therapeutic targets.
- miRNA negatively regulate gene expression and their expression profile used to classify tumors and play role in oncogenesis (mtr-17-92)
- In MPM, miRNA suggested as diagnostic markers, prognostic marker (mtR-29c), and potential therapeutic target (mtR-31)

Aim: To do comparative analysis of miRNA expression in MPM and normal mesothelial cell culture (HMC).

Methods:
- Cells:
  - 5 cell cultures they created from young patients without cancer
  - 5 cell cultures purchased from cell repositories
  - 3 cell cultures for positive controls for different genes
- RNA samples
  - Isolated from cell cultures and MPM
Differentially expressed genes (as > 2-folds) used for clustering analysis
- miRNA gene expression by microarray on 5 HMC and 5 MPM
- qRT-PCR used to validate 4 miRNAs
- Western blotting

Results:
- 151 miRNA differed between MPM and HCM and 22 were statistically significant (listed in Table 1).
- Chose 4 to validate mtR-15-5p, mtR-20a, mtR-92 and mtR-497 (don’t say why these 4 particularly).
- Same hybridized to human genome microarray to identify the target mRNA (don’t show data of this).
- Western blot for p21 expression (target for mtR17-92) which was normal in HMC and absent or reduced in MPM (And don’t describe any other proteins although in their discussion say WB confirms their 22 miRNA?)

Take home message
- Anticlimactic, seemed promising but we are left with list of miRNAs and no great idea about what they do except for mtR 17-92 with p21.
- I guess could be interesting to see if one could used this technology and these 22 miRNA to distinguish benign mesothelial proliferation from MPM.

8- Koh et al. Clinicopathologic Characteristics and Outcomes of Patients with Anaplastic Lymphoma Kinase-Positive Advanced Pulmonary Adenocarcinoma: Suggestion for an Effective Screening Strategy for These Tumors. JTO 2011; 6: 905-12

Background:
- ALK inhibitor crizotinib showing promise in the treatment of ALK+ NSCLC.
- Several studies suggest that patients with ALK+ NSCLC are younger, never smokers, adeno with signet ring cell features.
- ALK and EGFR are mutually exclusive.
- But no precise information on clinico-pathologic characteristics of ALK+ NSCLC?

Aim: Compare ALK+ to ALK- advanced NSCLC and determine effective screening strategy

Methods:
- Retrospective of all patients screened with ALK.
- All advanced metastatic or recurrent with detailed clinical information. EGFR testing and TTF-1 done.
- ALK testing IHC or FISH and then all IHC + cases had FISH.

Results:
- Total 221 patients and at time of ALK testing, 192 had undergone chemo, 113 EGFR TKI.
  - Signet ring features in 5, BAC in 22
  - EGFR in 135 patients with 46 showing mutation
  - TTF-1 + 76% (62/82).
  - 45 ALK+
- ALK+ patients were younger, all had TTF-1+ tumors and none had EGFR mutations. Smoking was not statistically significant and signet ring was marginal p=0.056.
ALK+ was not a significant prognostic marker, was a poor predictive factor for EGFR TKI response

**Take home message**

- Suggest to screen all adenocarcinoma TTF-1+, EGFR wild type, to exclude patients with objective response to EGFR TKI and not exclude smokers
- Nothing much new. I think some lack of statistic significance like for smoking can be due to small numbers.
- Like the idea of targeting specific populations to enrich for the genetic abnormality rather than just test anyone and anything but the numbers are not there yet.

### 9- Ding et al. Frequent loss of heterozygosity on chromosome 12q in non-small-cell lung carcinomas. Virchows Arch 2011; 458: 561-69

**Background:**
- Chromosomal aberrations are common in lung cancer resulting in mutations of TSG or amplification of oncogenes.
- Many chromosomes involved with losses or gains.
- Gain of 12q common in lung cancer
- In initial research the authors did using karyotypes of cell lines they saw abnormalities of chromosome 12 they wanted to explore.

**Aim:** LOH of chromosome 12 in 5 cell lines and 25 cases of NSCLC

**Methods:**
- G-banding and FISH on cell lines
- 25 cases of NSCLC and normal counterpart tissue
- LOH on all the above specimens using 14 microsatellites.
- Statistics to look at LOH of 12q and pathologic characteristics

**Results:**
- 68% of cases showed LOH of 12q.
- A lot of stats for 25 cases but the more frequent LOH the higher the stage and correlation between one microsatellite and histologic subtype.
- Also, correlated some microsatellites with gender and smoking history.

**Take home message**
- LOH in 12q frequent but not sure I would make too many other conclusions based on 25 cases even if p value <0.05. More to look at for the future and mostly good to know to look for TSG in these areas.


**Background:**
- Lack of effective screening for lung cancer.
- Lung cancer results from multi-step genetic abnormalities which are not only potentially useful for targeted therapies but also early diagnostic markers.
- One type of pre-invasive lesion is the sequence of hyperplasia, squamous metaplasia through dysplasia and carcinoma in-situ. Several molecular markers of this carcinogenetic sequence described.
Aim: To use IHC looking at different markers in the spectrum of histologic abnormalities from hyperplasia to CIS.

Methods:
- 67 biopsies comprising 89 lesions: 16 basal hyperplasia, 40 metaplasia, 33 dysplasia (7 mild, 7 moderate, 4 severe, 15 CIS).
- IHC for CK7, LP34 (CK5/6/18), chromogranin A, Ki67, p53, Her2, and EGFR.
  - For Ki-67, p53 and EGFR, intensity X % of + cells. 0-4 intensity scale so final score was 0-200% as low/neg, 201-300% as intermediate and 301-400% as high expression.
  - For Her2 same as breast.
- FISH for Her2, EGFR

Results:
- Basal cell hyperplasia was LP34 + and CK 7- in all cases as the squamous metaplasia and dysplasia thus authors concluded this was squamous? (English a little problematic)
- Chromo neg in all cases
- For Ki-67 high expression was seen in 6.25% of cases of hyperplasia, 22.5 of metaplasia and 57.6 of dysplasia with p=0.0002
- Same for p53 greater high expression in dysplasia (19% vs 22.5 vs 61) p=0.0007, and for EGFR IHC (0% vs2.5 vs 9.1) p=0.009.
- No Her2 overexpression by IHC.
- FISH showed mostly polysomy and number of FISH EGFR + cases greater in dysplastic cases (1 case vs 1 vs 11).

Take home message
- Although have achieved impressive p-values for different parameters, still enough overlap that it would be hard to use any of these markers to separate metaplasia vs dysplasia, although Ki-67 would have the most potential and we don’t know how mild dysplasia distinguishes itself from metaplasia as all cases of dysplasia were lumped together.

Review articles
1- Philipp Markart et al. Update in Diffuse Parenchymal Lung Disease 2010. AJRCCM 2011; 183: 1316-21
Review of all publications of 2010 regarding diffuse lung disease with emphasis on IPF/NSIP regarding etiology, diagnosis, prognosis and therapeutic trials. Also review of basic science of lung fibrosis. And small section on sarcoidosis and LAM. Good way of staying up to date!

Excellent review regarding testing of lung cancers with EGFR, k-ras and ALK. Sanja nicely addresses practical issues like amount of tissue, academic versus private practice, reference laboratories and so forth. Also goes over the proposed European work flow. She discusses reflex testing at her institution but doesn’t go into more details about their experience. Plus raised the issue of different mutation status between primary and met which would suggest waiting for the met and not testing the primary?
Review of the literature on EGFR and ALK testing in lung cancer. Authors suggest that reflex testing be done. But interestingly they site data from MSK that does reflex testing – 20% of 1831 patients with Stage I to IV tested had EGFR mutation. Stage I-III patients who underwent curative surgery, 78% (855 of 1097) had undergone reflex testing and 18% had EGFR mutation. Only 15% of these 1097 patients had recurrence, results of testing used. Don’t say how many of these 15% had the reflex testing nor the results and that means testing done for nothing in 85% of the time? At what cost? And no mention in this article about possibility of discrepant mutation and if treated with EGFR TKI what was the outcome….So I guess I don’t get the idea of reflex testing.

Case report
1- Conrado Abra˜o et al. Isolated Epithelioid Trophoblastic Tumor Mimicking Non-small Cell Lung Cancer. JTO 2011; 6: 966-7
Case report of an ETT to the lung in a 31 yo woman, last pregnancy 8 yr earlier presenting with ↑β-HCG and 2 normal curettage. The lung bx had been read as SQCC based on morphology and +p63. The H&E pictures good for ETT. And that would be a difficult differential dx!

Not clear if this is a case of UIP or other type of fibrotic lung with PCH like changes or truly primary PCH with secondary fibrosis (even Hcbg). Showed MMP-9 in macrophages (? non specific staining) and suggest that MMP-9 could have driven the fibrogenesis seen in this case of PCH.