PULMONARY PATHOLOGY JOURNAL CLUB
(October 2007 articles)
November 26, 2007

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Infections and infestations

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I. DISCUSSION ARTICLES

- **Purpose:** To expand previous observations by others (Dacic, 2004) suggesting that certain molecular alterations may be common to sclerosing hemangioma (SH) and non-mucinous bronchioloalveolar carcinoma (BAC).

- **Methods:**
  - 11 surgical specimens
  - IHC (cytokeratin [MNF116], EMA, TTF-1, EGFR, HER2/neu)
  - LOH, microdissecting “cuboidal surface” and “polygonal stromal” cells
  - FISH for EGFR and HER2 gene copy number
  - mutational analysis, sequencing DNA from microdissected “cuboidal surface” and “polygonal stromal” cells using same primers used for LOH/PCR

- **Results:**
  - Clinical features typical (median age 46.8 yrs, 10 women, mean size 3.1 cms, 1 with regional lymph node “mets”, all alive and well)
  - IHC stains typical, with positive EGFR in surface and stromal cells and negative HER2
  - FISH “negative” (*i.e.* diploid gene copy number) for EGFR and HER2 (<3% of cells showed EGFR trisomy in 2 cases)
  - no mutations in analyzed exons of EGFR (exons 18-21), HER2 (exons 19 & 20) and K-RAS (exon 2)
  - LOH informative in 9 cases; allelic losses in 5
    - 4 of 5 showed alterations in microsatellite markers related to p16 gene; 2 of these also showed alterations in Rb-related microsatellite markers
    - 1 showed alterations in the single TP53-related microsatellite marker tested
    - all 8 cases for which data were available showed identical LOH patterns in surface and stromal cells

- **Take-home message:** One more piece of evidence that both “surface” and ‘stromal” cell are neoplastic in sclerosing hemangiomas, despite my strong, personal aversion to the notion! Allelic losses occur, although these results differ in some important ways from those previously reported by Dacic et al in terms of frequency and targeted suppressor genes. This study showed allelic losses mainly in p16 and Rb while no LOH was demonstrated in FHIT-related markers tested. Interesting observations in that they parallel early events in carcinogenesis of a subset of BACs.

- **Purpose:** To use a novel internet based survey to test pathologist knowledge of/approaches to assessment of visceral pleural invasion (VPI) in lung carcinomas.

- **Methods:** An online quiz was distributed by email to an international pathology audience, comprising 3 sections
  1) introduction focusing on pathologist demographics
  2) questions regarding current practices
  3) 15 test cases for analysis (2-4 images, ≥ 1 VVG)

- **Results:**
  - Respondents
    - 103 pathologists from 22 countries (40.8% from USA)
    - 84.5% “academic”; 15.5% in training; 42.7% with subspecialty interest in pulmonary pathology
  - Assessment/reporting practices
    - 56.3% use templates for lung carcinomas; 98.1% specifically comment on VPI
    - elastic tissue stains used by 80.6% at least “occasionally”
  - Definitions (*i.e.* sufficient for diagnosis of VPI)
    - 10.7% - any tumor with visceral pleural puckering on gross exam
    - 13.6% - any tumor that abuts pleura “with no intervening alveoli on H&#”
    - 59.2% - tumors that extend into but not through visceral pleura
    - 25.2% - only tumors that extend to pleural surface
  - Quiz case performance
    - interobserver agreement 36.9% to 93.2% (highest for those in which majority agreed that VPI was present – 73.0%); kappa statistic 0.35 (“fair”)
    - participants highly variable in terms of likelihood that they would diagnose VPI (range 0.07 – 1.0; median 0.53)
    - interobserver agreement highest for pulmonary (74.9%) compared to “nonpulmonary” pathologists (58%)

- **Take home message:** The recent focus on visceral pleural invasion continues – what it means, how it’s defined, and how it’s assessed (see July and October 2007 journal clubs). Survey says, we may be all over the map but agree with each other more often than not! Another study intended to raise the question(s) without suggesting any answers.

- **Purpose:** To evaluate alpha-methylacyl CoA racemase (AMACR) expression in different histological subtypes of lung cancer and its correlation with survival.

- **Methods:** Tissue microarray blocks composed of 150 adenocarcinomas (ADK), 150 squamous cell carcinomas (SCC), 46 typical carcinoids (TC), 31 atypical carcinoids (AC), 28 large cell neuroendocrine carcinomas (LNEC), 72 small cell carcinomas (SCLC) and 90 normal lung cores were stained with P504S/p63 cocktail and P504S antibody alone. All finely granular cytoplasmic staining discernable at 200x was considered positive.

- **Results:** AMACR was positive in 56% of ADK, 22% of SCC, 72% of TC, 52% of AC, 70% of LNEC and 51% of SCLC. Normal lung parenchyma showed AMACR expression only in bronchial epithelium. An association was found between AMACR expression and stage I-II tumors, but AMACR expression did not correlate with age and sex. AMACR-positive SCLC had better disease-specific survival (19% vs 5% after 5 years, p=.04), but this survival advantage was seen only in patients with stage I-II disease. No association between AMACR and survival was found for ADK, SCC, AC and LNEC.

- **Take home message:** AMACR overexpression occurs in about half of lung carcinomas, less in SCC than other subtypes, and correlates with increased survival in stage I-II SCLC.

- **Purpose:** To evaluate the prognostic significance of synaptophysin (SYN) in stage I squamous cell carcinoma (SCC) and adenocarcinoma (ADK).

- **Methods:** An immunohistochemical study for SYN was performed on tumors of 318 patients with stage I SCC (162 cases) or ADK (156 cases) treated by surgery without adjuvant or neoadjuvant therapy. 10% or more of neoplastic cell staining was considered positive for SYN.

- **Results:** 27% of all tumors were SYN positive, including 21% of SCC and 33.3% of ADK. Patients with SYN positive tumors had a higher recurrence rate (50% vs 33.6%, p=.008), a shorter disease-free interval (40.24 vs 51.69 months, p=.01) and a lower 5 year survival rate (52.48% vs 72.68%, p=.0017, in subclass analysis statistical significance remains for SCC but not for ADK). Multivariate analysis showed that SYN positivity was an independent prognostic factor associated with a higher risk of death (HR=2.15, p=.0005).

- **Take home message:** SYN positive stage I SCC and ADK have a higher recurrence rate and lower survival rates.

- **Purpose:** To demonstrate morphologic and genotypic abnormalities in two siblings who died in infancy with “chronic pneumonitis of infancy” (CPI) attributable to mutations in a gene other than those encoding for surfactant proteins-B or C.

- **Methods:** Open lung biopsies were performed in two siblings at 2 and 7 weeks of age. Both were admitted to the NICU for tachydyspnea and cyanosis on the first day of life and required mechanical ventilation. They died 105 and 55 days after birth. Biopsy specimens were studied using conventional histology, IHC (surfactant protein-A, surfactant precursor protein-B, and mature surfactant protein-B), and EM. Mutational analysis was performed on DNA extracted from whole blood using primers for the *ABCA3* (ATP-binding cassette A3 transporter) gene.

- **Results:**
  - pictures look a lot like CPI (*i.e.* combination of NSIP, PAP and DIP, all rolled up in one)
  - surfactant proteins and precursors present by IHC
  - abnormal surfactant lamellar bodies on EM (‘fried-egg’ appearance – not like any eggs I’ve seen!)
  - both patients homozygous for a point mutation resulting in a Pro193Arg amino acid exchange in the ABCA3 protein; asymptomatic parents, sister and grandmother heterozygous; asymptomatic brother wild-type

- **Take home message:** There are a number of mutations that contribute to neonatal interstitial pneumonias, and not all specifically target surfactant proteins. Bottom line is that “classification” of neonatal interstitial pneumonias goes well beyond morphology – it’s in the genes! Think of this one if you get a lung biopsy that looks like CPI from an infant with normal surfactant protein levels.

- **Purpose:** To demonstrate potential value and limitations of CISH as an alternative to FISH for analyzing EGFR copy number amplification in NSCLC.

- **Methods:**
  - “specimens” (no details regarding specimen types) from 77 nonsmoking Taiwanese women with NSCLC treated by surgery alone (1999-2004)
  - FISH/CISH performed on each specimens using standard techniques
  - standard FISH definitions for segregating EGFR copy number into 4 different categories: no amplification, low genomic gain/low polysomy (< 4 copies of \( EGFR \) in > 40% of cells), high polysomy (≥ 4 copies of \( EGFR \) in > 40% of cells), and gene amplification (homogeneously staining regions with ≥ 15 copies in ≥ 10% of cells or a gene/chromosome ratio per cell of ≥ 2)
  - statistical analysis to demonstrate performance characteristics of CISH, using FISH as reference

- **Results:**
  - strong correlation between CISH scores and FISH copy numbers
  - low sensitivity (50%)/specificity (68%) for discriminating between nonamplified and low polysomy categories (a distinction of unknown/doubtful clinical value)
  - high sensitivity (89%) and specificity (89%) for discriminating between low polysomy/low amplification (FISH “negative”) and high polysomy/gene amplification (FISH “positive”)

- **Take home message:** CISH may be a more convenient and lower cost alternative to FISH should you need this assay in your shop!

- **Purpose:** To test the hypothesis that NSIP is a form of undifferentiated connective tissue disease (U-CTD), referring to patients with some clinical and serological features of CTD who fails to fulfill ACR criteria for disease classification. The majority of such patients do NOT evolve into a “differentiated” form of CTD.

- **Methods:** Retrospective analysis of patients consecutively enrolled in UCSF ILD database (JAN04 to NOV06), segregating patients with idiopathic interstitial pneumonia (IIP) into those with and those without U-CTD. Diagnostic criteria surprisingly loose: 1 or more symptom, including things as common as GERD, and 1 or more laboratory abnormality (e.g. ANA, RF, sedimentation rate, c-reactive protein, etc)

- **Results:**

<table>
<thead>
<tr>
<th></th>
<th>Undifferentiated CTD (n = 28)</th>
<th>No CTD (n = 47)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age at enrollment (range)</strong></td>
<td>54 years (33-69)</td>
<td>69 years (46-90)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Women (%)</strong></td>
<td>19 (68%)</td>
<td>11 (23%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Ever smokers</strong></td>
<td>12 (43%)</td>
<td>35 (75%)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>arthralgias/joint swelling</td>
<td>18 (64%)</td>
<td>6 (13%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Raynaud’s</td>
<td>17 (61%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>dysphagia</td>
<td>10 (36%)</td>
<td>2 (4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>skin changes (rash)</td>
<td>7 (25%)</td>
<td>1 (2%)</td>
<td>0.002</td>
</tr>
<tr>
<td>morning stiffness</td>
<td>5 (18%)</td>
<td>0</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Laboratory abnormalities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA positive</td>
<td>18/28 (64%)</td>
<td>2/33 (6%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RF positive</td>
<td>5/26 (19%)</td>
<td>2/29 (7%)</td>
<td></td>
</tr>
<tr>
<td>sed rate &gt; 2x normal</td>
<td>8/12 (67%)</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td><strong>HRCT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ground glass opacity</td>
<td>21 (75%)</td>
<td>4 (9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>honeycomb change</td>
<td>3 (11%)</td>
<td>37 (79%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Lung biopsy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSIP</td>
<td>15/18 (83%)</td>
<td>2/22 (9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UIP</td>
<td>1/18 (6%)</td>
<td>19/22 (86%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

- 24 (86%) of the U-CTD patients had at least two symptoms; 19 (67%) had at least three

- **Take home message:** NSIP on a surgical lung biopsy predicts for an “undifferentiated” CTD, but it remains unclear what it means to have an “undifferentiated” CTD

- **Purpose:** To characterized clinical, radiological and pathological features of asymptomatic ILD in patients from kindreds with familial IPF

- **Methods:** Retrospectively evaluated 164 patients from 18 kindreds evaluated at the NIH for familial IPF. Patients were categorized into 4 groups based on combination of symptoms (i.e. cough and/or dyspnea), self-reported history of ILD/IPF, and HRCT scan findings:
  1) Normal
  2) Nonspecific HRCT changes
  3) Asymptomatic ILD
  4) Familial IPF

- **Results:**
  - Clinical features

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 53; 32%)</th>
<th>Nonspecific HRCT changes (n = 59; 36%)</th>
<th>Asymptomatic ILD (n = 31; 19%)</th>
<th>Familial IPF (n = 21; 13%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever smoker</td>
<td>23%</td>
<td>25%</td>
<td>45%</td>
<td>67%</td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>35</td>
<td>40</td>
<td>46</td>
<td>67</td>
</tr>
<tr>
<td>Women</td>
<td>48%</td>
<td>59%</td>
<td>48%</td>
<td>52%</td>
</tr>
</tbody>
</table>

- DLco normal in asymptomatic ILD patients, but percent predicted value tended to be lower than that for normals/nonspecific changes (97% versus 105%)
- Percentage of BAL lymphocytes tended to be higher as did percentage of CD4 lymphs bearing CD38 or HLA-DR antigens (i.e. “activated”) compared to normals

- Histologic findings in 6 surgical lung biopsies
  - 3 patients with abnormal PFTs and HRCT=IPF – UIP
  - 3 patients with normal PFTs and “early” HRCT changes – HP (1), NSIP (1), cellular interstitial and organizing pneumonia

- **Take home message:** Asymptomatic IPF exists, and mostly it looks like UIP. Significance of biopsy showing other patterns uncertain, but might mean that some families with common environmental exposures have something other than familial “IPF”!
II. ARTICLES FOR NOTATION ONLY

Report of 5 South African HIV-positive patients who developed isolated soft tissue masses (chest wall – 3, arm, thigh) 6 to 12 months after treatment for cryptococcosis. Photomicrographs well worth seeing – an unusual tissue reaction resembling other lesions that have been lumped under the rather generic heading of “inflammatory pseudotumors”. Predominantly spindle cells that turn out to be mostly histiocytes with associated myofibroblasts. Lots of organisms so you’re unlikely to miss this, as long as you remember to look!

A review of 56 patients reported in the literature with severe COPD and invasive aspergillosis. Nice reference if you need one regarding epidemiology, clinical features, etc. There is no description of histologic findings in these cases although they make a short comment about potential role of lung biopsy in a population rarely well enough to survive one! Take home message is that this occurs in patients with COPD who are usually (77%) on steroids at the time of admission, and nearly all die (95%) despite treatment.

Confirms previous observations that induced sputums have reasonable yield in HIV-positive patients (although repeat induced sputum in this population doesn’t get you much) but are insensitive in HIV-negative patient. Frustration with paper is that it provides no details regarding either clinical status of HIV-negative group (i.e. were they all immunocompromised?) or number who ultimately were proven to have Pneumocystis?!

Description of histologic findings in 13 resected dirofilarial nodules. Authors note presence of eos, lymphs and plasma cells with high CD4:CD8 ratios, concluding that this is more than just infarction. Not sure this is anything new, but an updated reference the next time you want to know about dirofilariasis!

Nice study illustrating that patterns of viral attachment (PVA) are linked to pathogenesis and virulence of influenza infections. Human influenza virus A tends to target columnar epithelium of upper airways while avian influenza targets type II pneumocytes. Virulent and non-virulent forms of avian influenza have similar PVA, indicating that this alone is insufficient to explain differences in pathogenesis.

Case report – the title says it all!
Add CDC25B to the never ending list of candidate prognostic factors in NSCLC! CDC25 phosphatases are cell cycle regulators, and increased expression of this one has been reported in multiple tumor types including NSCLC. These authors used RT-PCR to show increased expression in 81 (45.8%) of 177 NSCLC patients with a significant association with expression of endothelin-1, intratumoral microvessels, and shorter overall and disease-free survivals.

Zeni et al. Macrophage expression of interleukin-10 is a prognostic factor in nonsmall cell lung cancer. Eur Respir J 2007; 30: 627-32
Speaking of prognostic factors in NSLC, another group from Italy looked at IL-10 staining in tumor associated macrophages (TAM) and tumor cells in 50 patients who underwent surgical excision and found that IL-10 staining in TAM (not tumor cells) was an independent predictor of higher tumor stage (II-IV) and therefore outcome. They claim that it may be the egg – hard to know if we’re looking at another chicken.

Authors analyzed 48 bronchial biopsies from ex-smokers and stained for TGFβ, Ki-67, MCM2 (another proliferation marker), and apoptosis/DNA fragmentation. In a study set that broke down into normal (7), metaplasia (25), dysplasia (8), and CIS (8) (3 invasive carcinomas included for comparison) they showed that with advancing neoplasia TGFβ and apoptoses diminish while proliferation rates increase.

Technically not a pathology paper but of interest to a group tasked with staging lung cancers. Using a large database and multivariate analysis the authors showed that, 1) advanced age at diagnosis, 2) male sex, 3) low socioeconomic status, 4) nonsurgical treatment, and 5) poor histologic grade (using a 4 grade system – and this is NOT from the Mayo Clinic!) were associated with increased risk of mortality. Location other than upper lobe and tumor size ≥4 cms were additional factors associated with increased mortality risk in patients with stage IB disease.

Another international multi-author position paper, this one focusing on acute exacerbation of IPF. No new data but a good overview with updated references should you need them. Perhaps most important contribution is proposed definition and diagnostic criteria that might be helpful in framing future studies. Definition is, “an acute, clinically significant deterioration of unidentified cause in a patient with underlying IPF.” Criteria focus on excluding other potential causes for acute respiratory failure in this setting. Not much on pathology, other than to acknowledge that most published cases have shown a combination of UIP and DAD (as so nicely illustrated in Figure 2, if I do say so myself!).


The only literature that seems to me more rapidly expanding and bewildering than papers touting prognostic factors/markers in NSCLC is the science of regulatory molecules in lung fibrosis! This is a nice review with a really pretty picture that you can download as a PowerPoint slide, summarizing current knowledge of pathogenesis. The cool thing is that I almost understand it while I read it, but within 10 minutes I remain no smarter than I was at baseline!


Yet another review of smoking-induced ILD! Not much new here, although authors continue to argue for separating RBILD and DIP (despite their own previous contributions indicating difficulty in separating one from the other in any individual case).


Second case report on today’s menu. Patient was young (17), and yes he DID survive!