# Table of Contents

**Discussion articles**


**Articles for notation**

**Neoplastic diseases**


**Page 11** Archives of Pathology & Laboratory Medicine 2008; 132

- Allen T. Pulmonary neoplasia. 1053-4.


- Beasley MB. Immunohistochemistry of pulmonary and pleural neoplasia. 1062-72.

- Dacic S. Pulmonary preneoplasia. 1073-8.

- Dishop M and Kuruvilla S. Primary and metastatic lung tumors in the pediatric population: A review and 25-year experience at a large children’s hospital. 1079-1103.

- Litzky L. Pulmonary sarcomatous tumors. 1104-17.

- Butnor K. Avoiding underdiagnosis, overdiagnosis, and misdiagnosis of lung carcinoma. 1118-32.

- Borczuk A. Benign tumors and tumorlike conditions of the lung. 1133-48.

- Guinee D and Allen T. Primary pleural neoplasia: Entities other than diffuse malignant mesothelioma. 1149-70.

- Allen T. Pulmonary Langerhans cell histiocytosis and other pulmonary histiocytic diseases: A review. 1171-81.

**Non-neoplastic diseases**


I. DISCUSSION ARTICLES

- **Purpose:** To test the ability of a specialized new technology – the CTC-chip – to detect circulating tumor cells in patients with NSCLC to permit mutational analysis of epidermal growth factor receptor (EGFR) gene.

- **Methods:**
  
  A test of the reliability and utility of new technology: CTC-chip and SARMS real-time PCR assay

  Analyzed EGFR mutational status of tumors by capturing circulating tumor cells (CTCs) using the CTC-chip; isolated DNA analyzed by Scorpion Amplification Refractory Mutation System (SARMS) assay, an allele-specific PCR amplification system designed to detect exon 19 deletions, insertions in exon 20, mutations in codon 719, and T790M mutants (associated with resistance to tyrosine kinase inhibitor therapy)

  1. Collected blood on 23 patients with EGFR mutant NSCLCs and 4 with EGFR wild-type tumors, and quantified number of CTCs
  2. SARMS validation portion: Compared SARMS assay with direct sequencing on paraffin-embedded tumor-biopsy specimens (26 EGFR mutant tumors and 8 wild-type)
  3. Applicability of SARMS with CTC-chip among 20 patients with tumor-biopsy specimens and peripheral blood
  4. Comparison of SARMS + CTC-chip vs. SARMS + free plasma DNA among 18 patients with EGFR mutant tumors and 3 controls with wild-type tumors
  5. Serial analysis of CTCs and tumor genotype in 4 patients treated with Gefitinib

- **Results:**

  1a. CTCs identified in all patients, with a similar number in EGFR mutant vs. wild-type tumors
  1b. The number of CTCs *at a single point in time* did not correlate with tumor burden
  2a. SARMS assay had 96% sensitivity, 100% specificity for detection of EGFR mutations in tumor-biopsy samples (gold standard – direct sequencing)
  2b. T790M mutation associated with decreased progression-free survival
  3. SARMS + CTC-chip identified EGFR mutants in 19/ 20 cases (95% sensitivity)
  4. SARMS + CTC-chip sensitivity 94% vs. SARMS + free plasma DNA sensitivity 33-39%
  5a. Gefitinib treatment associated with profound decrease in numbers of CTCs
  5b. Clinical progression was associated with an increase in CTCs
  5c. Tumor burden correlated with CTC number in patients who were followed serially during treatment

- **Take-home message:** Capture and molecular analysis of circulating tumor cells promises to be a powerful new technique to gauge clinical response, monitor tumor genotype, and potentially modify/ tailor therapy in patients with lung cancer (and probably many other carcinomas!!)

This is a LOUSY article: bad science, bad writing, bad translation. Don’t read it!

- **Purpose:** Authors sought to determine EGFR mutation status in lung mucoepidermoid carcinomas (MECs).

- **Methods/ Results:**
  (a) Performed EGFR mutational analysis (direct sequencing, copy number by FISH) in a case of pulmonary MEC in a 46 yo F – no mutation. Despite this, patient’s tumor shrank on gefitinib (a specific type of tyrosine kinase inhibitor) treatment.

  (b) Pulled 6 cases of “lung MEC” (we have reason to doubt the diagnoses!) from 2000-2006, three of which showed EGFR mutations (analyzed by 3 modalities: direct sequencing + FISH + IHC).

- **Take-home message:**
  (a) Some fraction (??) of lung MECs harbor EGFR activating mutations.

- It’s possible to get bad science published in a peer-reviewed journal.

- **Purpose:** Another paper using a molecular technique (LOH) to compare genotypes between histologically disparate components of a biphasic lung carcinoma, in this case a combined small/large cell NEC and adenocarcinoma

- **Methods:**
  - 2.5 cm peripheral combined ca (70% adca/30% small cell LCNEC) in smoker (T1NOMO)
  - standard IHC (TTF, CDX2, CRG, SYN, CD56, NSE, Ki-67)
  - LOH for 40 microsatellite markers on 13 chromosomes
  - fractional allelic loss index (FAL): # markers with LOH/# informative markers

- **Results:**
  - histology a bit confused, describing a combination of LCNEC (“A” and “B”) and small cell carcinoma in neuroendocrine component of an otherwise typical peripheral adca (pretty much chose to ignore this part of the description assuming that it’s more or less a combined SCLC!)
  - IHC as predicted with expression of neuroendocrine proteins restricted to areas with NE histology and TTF common to both
    - higher mitotic rates and Ki-67 labeling indices in NE components
  - 30 informative markers
    - 22 showed concordant allelic losses in adca and SCLC components
    - 9 showed concordant allelic losses in “all four components” although no confidence that there are really any more than two components in this tumor
  - FAL index higher in NE components (0.60 in SCLC) compared to adca (0.33)

- **Take-home message:** Histologically heterogeneous lung carcinomas reflect heterogeneous differentiation in a tumor derived from a common origin/stem cell, which is pretty much what I was taught in the early 1980s!

- **Purpose:** To test the specificity of histology in separating chronic hypersensitivity pneumonia (HP) from those with UIP/IPF.

- **Methods:**
  - surgical lung biopsies from Mayo Clinic patients (JAN97 – JUN05) with chronic HP diagnosed on basis of clinical and/or histologic criteria
    - clinical criteria: chronic (≥ 3 months) symptoms, crackles, CxR/CT = diffuse lung disease, hx of exposure and/or pos ppt antibodies, no other identifiable cause for lung disease
    - histologic criteria: bronchiolocentric CIP and/or chronic bronchiolitis, peribronchiolar granulomatous inflammation confined to interstitium
  - controls = surgical lung biopsies from patients with UIP/IPF or idiopathic NSIP
  - slides stripped of identifiers, randomly coded, and aggregated as unique specimens independent of previous diagnosis
  - slides reviewed blindly at double headed scope by 2 authors (Sylvain and me) with no knowledge regarding numbers of patients or specimens in study and control groups
    - tabulated histologic features as present or absent (bronchiolocentric CIP, CIP w “UIP pattern”, fibroblast foci, honeycomb change, lymphoid hyperplasia, bronchiolitis, peribronchiolar metaplasia, granulomas, organizing pneumonia)

- **Results:**

  **Clinical findings**

<table>
<thead>
<tr>
<th>F:M</th>
<th>12:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean age (range)</td>
<td>58.7 yrs (46 – 79 yrs)</td>
</tr>
<tr>
<td>ever smokers</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>median symptom duration (range)</td>
<td>18 months (4-130 mos)</td>
</tr>
<tr>
<td>antigenic exposure identified</td>
<td>10</td>
</tr>
<tr>
<td>– history of exposure</td>
<td>9 (birds-5, mold-2, thermophilic bact-2)</td>
</tr>
<tr>
<td>– ppt antibodies</td>
<td>4 (avian-3, M faeni-1)</td>
</tr>
<tr>
<td>outcome (n=14)</td>
<td>7</td>
</tr>
<tr>
<td>alive &amp; well, improved, stable</td>
<td>4</td>
</tr>
<tr>
<td>worse</td>
<td>3 (includes both patients with UIP only on bx – see below)</td>
</tr>
<tr>
<td>dead</td>
<td></td>
</tr>
</tbody>
</table>

- **Histological findings**
  - 10 patients/22 specimens = HP in all samples
  - 2 patients/5 specimens = “discordant” HP (HP + UIP + UIP; HP + emphysema)
  - 3 patients/4 samples ≠ HP (UIP; UIP + UIP; NSIP)
  - findings in 24 biopsies diagnostic of HP

  | chronic bronchiolitis | 23 (96%) |
  | lymphoid hyperplasia | 19 (79%) |
  | peribronchiolar metaplasia | 13 (54%) |
  | organizing pneumonia | 10 (42%) |
  | isolated MNGCs/loose/well formed granulomas | 17 (71%)/14 (58%)/2 (8%) |
  | CIP | 20 (83%) |
  | honeycomb change | 5 (21%) |
  | fibroblast foci | 12 (50%) |

- **Take-home message:** SLBx useful but imperfect in separating patients with HP from those with UIP/IPF. Beware of cases showing UIP with associated bronchiolitis/peribronchiolar metaplasia (although once it looks like UIP it tends to act like it!).

- **Purpose:** To determine the cause of death (COD) in patients with IPF (since relatively few studies have examined COD in IPF patients) and document pathologic findings at post-mortem in IPF patients. This will help (a) elucidate the natural history of IPF and (b) help focus clinical and research efforts to improve survival.

- **Methods:** 42 patients with IPF who underwent post-mortem at Mayo Clinic (Rochester) (1996-2004)
  
  a) Diagnosis established by post-mortem evidence of UIP
  b) Excluded those with connective tissue disease, exposure to “fibrogenic drugs or environmental agents”
  c) 38 complete post-mortems, 3 heart + lung only, 1 lung only
  d) COD assigned by consensus of 2 authors based on all available data

- **Results:** Immediate COD
  
  a) Respiratory - 27 cases (64%)
     - Acute exacerbation (DAD + UIP) – 12 (29%)
     - Gradual progression – 5 (12%)
     - Infectious pneumonia – 8 (19%)
  b) Cardiovascular – 9 cases (21%)
     - Arrhythmia (3) + MI (3)
     - Cor pulmonale – 2
       - However, 17 cases (40%) showed histologic evidence of pulmonary hypertension, of which 12 had undergone echocardiography. 11 of these (92%) showed pulmonary hypertension (by echocardiography).
  c) Other – 6 cases (14%)
     - Multiorgan failure, trauma, ARF, anoxic encephalopathy
  d) Also of note:
     - IPF clinically unsuspected in 9 cases (21%)
     - Of these, acute exacerbation immediate COD in 4, gradual progression in 1

- **Take-home message:**
  
  a) IPF itself is the immediate COD in substantial, albeit not majority, of cases (40%) of IPF patients undergoing post-mortem.
  b) Acute exacerbation of IPF was the single most common COD in IPF patients undergoing post-mortem.
  c) Clinically occult IPF was frequent finding, and frequently presents as acute exacerbation of IPF.
  d) Pulmonary hypertension is a frequent contributing COD in IPF patients undergoing post-mortem.

- **Purpose:** To bring to the attention of the surgical pathology community a rare manifestation of extrapulmonic infection with *Pneumocystis jiroveci*.

- **Methods:**
  - retrospective descriptive study of 6 cases drawn from the files of a single South African academic medical center (Nelson R Mandela School of Medicine)

- **Results:**
  - all 6 patients presented with hearing loss and a mass/polyp ± otalgia (4), otorrhea (2), and pruritis (3)
    - external auditory canal involved in all; middle ear in 2
  - biopsies showed,
    - prominent granulation tissue + focal ("elusive") foamy exudate (2)
    - mainly foamy exudate (4); 1 with granulomatous reaction
  - all patients diagnosed with AIDS after ear biopsy
  - 2 patients died of pulmonary TB (2 and 4 weeks), 3 lost to follow-up (1 with PCP), and 1 NED at 4 years

- **Take-home message:** Before you complain about what a nasty and uninformative specimen that ear thing is, you better look carefully for *Pneumocystis*!
II. ARTICLES FOR NOTATION ONLY
Neoplastic diseases

- **Purpose:** To test, 1) feasibility of obtaining sufficient tissue for immunohistochemical studies by EBUS-TBNA, and 2) value of IHC in identifying patients responsive to platinum based combination chemotherapy.

- **Methods:** retrospective descriptive case study of patients with diagnoses of pN2 NSCLC established on basis of EBUS-TBNA at single institution (Chiba University, Japan) from JUL04 – APR06
  
  - huge selection bias in terms of addressing first hypothesis if you define study set as those with a diagnostic procedure; no idea how many had falsely negative/non-diagnostic EBUS-TBNA over same time period.
  - 36/67 (54%) patients with positive EBUS-TBNA had sufficient tissue for IHC (so in every respect this belongs in the Results and not the Methods!)
  - IHC stains for,
    - Rb pathway proteins (pRb, cyclin D1, p16\(^{INK4A}\)) – negative staining/expression (<10% of tumor cells) is abnormal for pRb and p16; positive staining (>10%) is abnormal for cyclin D1
    - p53 pathway proteins (p53, p21\(^{Waf1}\)) – positive staining/expression (>10% of tumor cells) is abnormal for p53; negative staining/expression (<10%) is abnormal for p21
    - Ki-67 – labelling index >20% considered “abnormal” or “positive” result
  - assessed complete/partial response using same Response Evaluation Criteria in Solid Tumors (RECIST) guidelines mentioned in first paper.

- **Results:**
  - 19 (52.8%) adcas/17 (47.2%) squamous cell carcinomas
  - 1 complete + 12 partial responses (response rate = 46.4%)
  - all cases showed abnormal expression of at least one protein

<table>
<thead>
<tr>
<th></th>
<th>Frequency of Abnormal Expression (see above definitions of “abnormal” staining)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pRb</td>
</tr>
<tr>
<td>adca (19)</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>sq cell ca (17)</td>
<td>8 (47.1%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>13 (36.1%)</td>
</tr>
</tbody>
</table>

adca vs sq cell ca: p = *0.042 and †0.007

- both p53 (p=0.002) and p21 (p=0.011) expression were statistically associated with treatment response in univariate analysis (p53 = non-response; p21 = response), but only p53 survived multivariate analysis (p=0.021) and was also associated with risk of disease progression (p=0.044)

- **Take-home message:** EBUS-TBNA can get you tissue for IHC in about half of patients with a positive result, and the value of staining remains unclear but p53 may (or may not) predict treatment response/disease progression.

- **Purpose:** Glypican-3 (GPC3) is part of a family of extracellular membrane-bound proteins whose expression is downregulated after birth. The GPC3 gene is on Xq26 and when functionally inactivated results in an X-linked syndrome (Simpson-Golabi-Behmel) associated with increased incidence of embryonal tumors suggesting it may have tumor suppressor function. Authors set out to expand previous studies looking at expression and potential role of GPC3 in lung carcinogenesis.

- **Methods:**
  - IHC applied to TMA containing 107 samples (97 ultimately included in study set given criteria of having at least 2 cores for interpretation)
  - 143 fresh samples (58 overlapped with IHC cohort) for GPC3 mRNA and RAS sequencing

- **Results:**
  - results of IHC highly correlated with mRNA levels (normal tissues GPC3 negative)
  - 22 (23%) of carcinomas IHC-positive, especially squamous cell carcinomas (17/31 [55%] sq cell cas vs 5/59 [8%] adcas)
  - RAS mutations in 30%, strongly associated with adca histology (42/101 [42%] of adcas vs 6/57 [11%] of sq cell cas) and inversely associated with GPC3 expression (GPC3 positive in 18/70 [26%] wild type vs 4/27 [15%] mutated tumors) and mRNA levels
  - GPC3 mRNA levels correlated with smoking within each histological category (adca + smoking = ↓mRNA; sq cell ca + smoking = ↑mRNA); similar analysis using protein expression not significant because of small numbers (n = 53)
  - stage was only predictor of outcome (*i.e.* neither GPC3 expression nor mRNA levels were associated with survival).

- **Take-home message:** Yet another protein to add to an increasingly complex map of potential players in lung carcinogenesis, this one associated with histology and RAS expression but not to outcome. When it comes to outcomes, pretty hard to beat tumor stage!

- **Purpose:** Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is involved in mediation of apoptosis via 4 membrane bound receptors, 2 agonistic (death receptors 4 and 5 – DR4, DR5) and 2 antagonistic decoys (R3, R4). Deregulation of the TRAIL pathway has been implicated as a mean of evading apoptosis in many human tumors including NSCLC. The authors set out to investigate prevalence and possible significant of the proapoptotic receptor DR5 in NSCLC.

- **Methods:**
  - tumor from 146 stage I (118) & II (28) NSCLC patients (1996-1999)
    - 69 (47.3%) adca (including 10 BACs), 61 (41.8%) sq cell ca, 16 (11.0%) LCC
    - 17 with regional LN mets
    - “precursor” lesions in some (10 squamous metaplasia, 3 low grade dysplasia, 9 ca in-situ)
  - constructed TMAs (3-4 donor cores)
  - DR5 IHC assessed as % of cells staining by 2 pathologists with good agreement; average score assigned; 100% staining = “high protein expression”

- **Results:**
  - DR5 “expression” in non-neoplastic bronchial/alveolar epithelium, smooth muscle cells, fibroblasts and endothelial cells (i.e. code for HIGH BACKGROUND making interpretation suspect, at best – difficult to appreciate in black and white printouts, but check out the color photos in the online version)
  - DR5 staining (cytoplasmic) seen in pretty much all NSCLCs (mean value of DR5+ cells 90.9%±17.9%; median 100%), and was higher in more poorly differentiated tumors
  - text says that 98 (67.1%) of cases showed “high” DR5 expression, but according to all of their tabulated data the number is actually 78 (53.4%) – see below!!

  **EDITORIAL COMMENT:** Probably not a big deal, but always marvel at the things that get by investigators and reviewers – kinda hard to trust anything else they say when it comes to numbers.

<table>
<thead>
<tr>
<th></th>
<th>high DR5 expression</th>
<th>low DR5 expression</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>adenocarcinoma</td>
<td>33 (56%)</td>
<td>26 (44%)</td>
<td>.74</td>
</tr>
<tr>
<td>BAC</td>
<td>2 (20%)</td>
<td>8 (80%)</td>
<td><strong>0.045</strong></td>
</tr>
<tr>
<td>sq cell ca</td>
<td>31 (51%)</td>
<td>30 (49%)</td>
<td>.62</td>
</tr>
<tr>
<td>LCC</td>
<td>12 (75%)</td>
<td>4 (25%)</td>
<td>.11</td>
</tr>
<tr>
<td>well differentiated</td>
<td>4 (33%)</td>
<td>8 (67%)</td>
<td>.23</td>
</tr>
<tr>
<td>moderately differentiated</td>
<td>38 (47%)</td>
<td>43 (53%)</td>
<td>.096</td>
</tr>
<tr>
<td>poorly differentiated</td>
<td>36 (68%)</td>
<td>17 (32%)</td>
<td>.010</td>
</tr>
<tr>
<td>squamous metaplasia</td>
<td>4 (40%)</td>
<td>6 (60%)</td>
<td>ND</td>
</tr>
<tr>
<td>low grade dysplasia</td>
<td>0</td>
<td>3 (100%)</td>
<td>ND</td>
</tr>
<tr>
<td>ca in-situ</td>
<td>5 (56%)</td>
<td>4 (44%)</td>
<td>ND</td>
</tr>
<tr>
<td>LN mets (17)</td>
<td>13 (76%)</td>
<td>4 (24%)</td>
<td>NS</td>
</tr>
</tbody>
</table>
  - adca (4), sq cell ca (9), LCC (4)

- DR5 showed weak correlation with overall survival, but not disease-free survival, in univariate analysis but failed to emerge as independent prognostic factor in multivariate analysis (stage, age, vascular invasion were the winners), except in smokers

- **Take-home message:** Don’t think we’ll have to add DR5 to our rapidly expanding list of putative prognostic markers, although authors suggest that it may someday be a target for novel therapies, which is pretty much part of the conclusions of every similar paper these days!

- **Purpose:** To describe what may be one of the unluckiest patients yet reported in the pulmonary pathology journal club, a 62-year-old high school teacher who had both a peripheral adenocarcinoma and a diffuse pleural mesothelioma! Non-smoker with no occupational asbestos exposure.

- **Methods:** Usual IHC stains

- **Results:** Adca looked and stained like adca. Mesothelioma looked and stained like mesothelioma. Asbestos fiber counts low, failing to suggest exposure above ambient/environmental.

- **Take-home message:** Stuff happens.
Archives of Pathology & Laboratory Medicine 2008; 132

- Allen T. Pulmonary neoplasia. 1053-4.
- Beasley MB. Immunohistochemistry of pulmonary and pleural neoplasia. 1062-72.
- Dacic S. Pulmonary preneoplasia. 1073-8.
- Dishop M and Kuruvilla S. Primary and metastatic lung tumors in the pediatric population: A review and 25-year experience at a large children’s hospital. 1079-1103.
- Litzky L. Pulmonary sarcomatous tumors. 1104-17.
- Butnor K. Avoiding underdiagnosis, overdiagnosis, and misdiagnosis of lung carcinoma. 1118-32.
- Borczuk A. Benign tumors and tumorlike conditions of the lung. 1133-48.
- Guinee D and Allen T. Primary pleural neoplasia: Entities other than diffuse malignant mesothelioma. 1149-70.
- Allen T. Pulmonary Langerhans cell histiocytosis and other pulmonary histiocytic diseases: A review. 1171-81.
Non-neoplastic diseases


- **Purpose:** 8 authors describe an unusual cystic lung lesion in a single patient using conventional case report format.

- **Results:**
  - 5-year-old previously healthy girl presented with “upper respiratory tract infection” and was discovered to have a left sided intrathoracic mass with mediastinal shift; she underwent left upper lobectomy
  - amazingly cool looking 13.5 cm cystic mass with multiple cartilaginous islands embedded within a rather nondescript mature fibroadipose tissue
  - cystic spaces lined by TTF1-positive epithelium
  - trisomy 8 detected in 6/17 metaphases by conventional cytogenetics, and in 18% of tumor nuclei by FISH compared to 13% in nuclei from normal lung

- **Take-home message:** There sure are a lot of weird cystic things when it comes to pediatric lung disease!


- **Purpose:** To describe 3 patients with narrowing/obliteration of bronchial lumens attributed to anthracofibrosis linked to mixed dust exposure. Anthracofibrosis is defined as narrowing or obliteration of the bronchial lumen of any cause (e.g. TB, tumor, etc) associated with black pigmentation of overlying mucosa. Not really a pathology paper in that there are no gross or histopathological illustrations of pathology specimens so not sure how or whether you’re ever going to use this one!

- **Take-home message:** Add mixed dust exposure to list of etiologies for this unusual and clinically/endoscopically defined condition!