## PULMONARY PATHOLOGY JOURNAL CLUB
(February 2009 articles)

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I. DISCUSSION ARTICLES

- **Background:**
  - Pulmonary adenocarcinoma with intestinal differentiation
    - Typical immunophenotype
      - CK7 +, CK20 -/+ , TTF-1 +/−, CDX-2 −/+  

- **Purpose:**
  - To report a case of pulmonary adenocarcinoma with intestinal differentiation with negative CK7 expression.

- **Results:**
  - 51-year-old woman with a solitary 3.3-cm mass in the left lower lobe.
  - Additional clinical investigation revealed no evidence of tumor elsewhere.
  - She underwent left lower lobectomy with mediastinal lymphadenectomy.
  - Histological examination revealed tall columnar cells without goblet cell differentiation arranged in a cribriform and acinar pattern with extensive central necrosis.
  - Metastatic carcinoma was present in multiple hilar lymph nodes. Mediastinal lymph nodes were negative.
  - Immunohistochemical stains:
    - CK7 −, CK 20 +, TTF-1 − , CDX-2 +

- **Take-home message:**
  - This is the first description of a pulmonary adenocarcinoma with intestinal differentiation with negative CK 7 expression.

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**Table 1**
Reported Cases of Primary Pulmonary Adenocarcinoma With Intestinal Differentiation

<table>
<thead>
<tr>
<th>Reference/Sex/Age (y)</th>
<th>Size (cm)/Site</th>
<th>CC-like Component (%)</th>
<th>Immunohistochemical Results</th>
<th>Stage</th>
<th>Clinical Follow-up (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inamura et al11 M</td>
<td>5.0/RUL 50</td>
<td>+ + − + −</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N1M1</td>
<td>D (82)</td>
</tr>
<tr>
<td>M</td>
<td>4.0/LUL 60</td>
<td>+ + − + +</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N1M2</td>
<td>D (47)</td>
</tr>
<tr>
<td>M</td>
<td>2.6/RLL 70</td>
<td>+ P+ − − + P+</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T1N0M0</td>
<td>A (43)</td>
</tr>
<tr>
<td>M</td>
<td>3.4/RLL 50</td>
<td>+ P+ − P+</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N0M0</td>
<td>A (43)</td>
</tr>
<tr>
<td>M</td>
<td>2.3/RLL 80</td>
<td>+ − − P+ P+</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T1N0M0</td>
<td>A (30)</td>
</tr>
<tr>
<td>M</td>
<td>1.7/RLL 80</td>
<td>+ − − − −</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N1M1</td>
<td>A (12)</td>
</tr>
<tr>
<td>M</td>
<td>3.9/LUL 80</td>
<td>+ − − − −</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N1M0</td>
<td>A (12)</td>
</tr>
<tr>
<td>Yousem10 F/74</td>
<td>3.6/RUL NA</td>
<td>+ − − − −</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N1M0</td>
<td>DOD (26)</td>
</tr>
<tr>
<td>F/70</td>
<td>1.7/RUL NA</td>
<td>+ − − − −</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N1M0</td>
<td>DOD (18)</td>
</tr>
<tr>
<td>M/62</td>
<td>6.6/RUL NA</td>
<td>+ − − P+</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N0M0</td>
<td>D (5)</td>
</tr>
<tr>
<td>F/63</td>
<td>1.5/RUL NA</td>
<td>+ − − − −</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T1N0M0</td>
<td>ANED (7)</td>
</tr>
<tr>
<td>F/73</td>
<td>7.0/LUL NA</td>
<td>+ − − − −</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N0M0</td>
<td>ANED (3)</td>
</tr>
<tr>
<td>F/57</td>
<td>2.0/RUL NA</td>
<td>+ − − − −</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N0M0</td>
<td>ANED (2)</td>
</tr>
<tr>
<td>M/69</td>
<td>2.5/RLL Predominant</td>
<td>+ − − + NA</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T1N0M0</td>
<td>NA</td>
</tr>
<tr>
<td>Present case</td>
<td>3.3/LLL 100</td>
<td>+ − − − +</td>
<td>CK7 + CK20 + TTF-1 − CDX-2 +</td>
<td>T2N1M0</td>
<td>ALR (10)</td>
</tr>
</tbody>
</table>

A: alive; ALR, alive, local recurrence; ANED, alive, no evidence of disease; CC, colorectal carcinoma; CK, cytokeratin; D, died; DNED, died, no evidence of disease; DOD, died of disease; LLL, left lower lobe; LUL, left upper lobe; NA, not available; P, patchy; RLL, right lower lobe; RUL, right upper lobe; TTF, thyroid transcription factor; +, positive; −, negative.

* When reported.

- **Background:**
  - Evidence-based medicine (EBM) was defined in the early 1990s as “the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients”.
  - Similar concepts have recently been described in pathology literature and have been designated as evidence-based pathology (EBP).

- **Purpose:**
  - To understand the probabilities that particular pathologic features will be present or absent in a case of pulmonary carcinoid tumor on FS.

- **Methods:**
  - 6 steps:
    - Formulating specific questions.
    - Querying the literature for answers and classification of information into “levels of evidence”.
    - Review of the experience at Cedars Sinai Medical Center.
    - Statistical analysis.
    - Selection of evidence-based pathologic features.
    - Testing for the clinical use of the criteria with likelihood ratio (LR=sensitivity/1-specificity) above 5 and LR below 0.5 for the diagnosis of carcinoid tumors.

- **Results:**
  - Frozen section diagnosis was requested on 58 typical carcinoids and 8 atypical carcinoids from 2405 patients that underwent frozen section diagnosis at Cedars-Sinai Medical Center from 2002 to 2007.
  - The deferral and error rate for carcinoid tumors was 4.13% and 7.5%, respectively, and resulted in 4 unnecessary lobectomies and 2 second thoracotomies.
  - The most common errors included misdiagnoses as lymphoma, squamous carcinoma or metastasis from breast carcinoma.
  - Thirty one pathologic features were evaluated in the 66 carcinoid tumors and 10 frozen sections each of lymphoma, squamous cell carcinoma, and metastatic breast carcinoma.
  - Seven pathologic features were significant by chi square test at P > .05.
  - Positive likelihood ratios identified 11 pathologic features that were useful for the diagnosis of carcinoid tumor from other neoplasms.
  - The applicability of the 11 pathologic features was tested with a group of pathologists, resulting in significant improvement in diagnostic accuracy as measured by pre and posttests.

- **Take-home message:**
  - The significant improvement in the posttest results suggests that the 11 pathologic features identified in this study may be clinically useful.

- **Purpose:**
  - To identify diagnostic criteria that would help distinguish primary lung adenocarcinoma from metastatic breast carcinoma by FS, using the systematic approach favored by proponents of evidence-based pathology.

- **Method:**
  - 129 FSs from 121 patients with a pulmonary nodule and a history of breast cancer was reviewed.

- **Results:**
  - The pretest odds ratio of primary pulmonary carcinoma/metastatic breast carcinoma was 2.6.
  - The incidence of 12 histopathologic features was assessed in a “training set” composed of 20 FSs, 10 with primary lung adenocarcinoma and 10 with metastatic breast cancer.

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**Table 1**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Posttest Odds Ratio (Lung/Breast)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acini</td>
<td>34</td>
<td>.0001</td>
</tr>
<tr>
<td>Lepidic growth</td>
<td>17</td>
<td>.055</td>
</tr>
<tr>
<td>Nuclear pseudoinclusions</td>
<td>14</td>
<td>.015</td>
</tr>
<tr>
<td>Scar</td>
<td>11</td>
<td>.033</td>
</tr>
<tr>
<td>Macronucleoli</td>
<td>3.02</td>
<td>.213</td>
</tr>
<tr>
<td>Subpleural location</td>
<td>0.20</td>
<td>.168</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>0.06</td>
<td>.174</td>
</tr>
<tr>
<td>Papillary architecture</td>
<td>Infinity</td>
<td>.163</td>
</tr>
<tr>
<td>Comedonecrosis</td>
<td>0.115</td>
<td>.0006</td>
</tr>
<tr>
<td>Solid nests</td>
<td>0.0087</td>
<td>.002</td>
</tr>
<tr>
<td>Trabecular architecture</td>
<td>0</td>
<td>.002</td>
</tr>
<tr>
<td>Cribriform architecture</td>
<td>0</td>
<td>.002</td>
</tr>
</tbody>
</table>

* Features in italics favor primary lung carcinoma; features in bold favor metastatic breast carcinoma.

- **Take-home message:**

**Table 2**

<table>
<thead>
<tr>
<th>Participants</th>
<th>Pretest</th>
<th>Posttest</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residents (n = 5)</td>
<td>82</td>
<td>92</td>
<td>.108</td>
</tr>
<tr>
<td>Fellows (n = 4)</td>
<td>79</td>
<td>88</td>
<td>.116</td>
</tr>
<tr>
<td>Attending physicians (n = 10)</td>
<td>77</td>
<td>89</td>
<td>.005</td>
</tr>
<tr>
<td>All participants (n = 10)</td>
<td>78</td>
<td>89</td>
<td>.001</td>
</tr>
</tbody>
</table>

* Data are given as percentage of correct answers.

- **Background:**
  - Patients with advanced pulmonary adenocarcinoma exhibiting overexpression or mutation of epidermal growth factor receptor tend to respond better to targeted therapy with tyrosine kinase inhibitors such as gefitinib and erlotinib.
  - There is no consensus regarding how these neoplasms should be routinely tested for epidermal growth factor receptor (EGFR).

- **Purpose and Methods:**
  - To test 100 pulmonary adenocarcinomas from patients with stage III or IV disease for EGFR abnormalities using IHC, PCR (deletions in exons 19 and 21 point mutation L858R) and FISH.
  - To compare the results.

- **Results:**
  - Kappa statistics yielded poor concordance between the results of the EGFR tests.
  - Strong membranous immunoreactivity in more than 90% of the tumor cells was found to correlate with amplification or polysomy as detected by FISH.

- **Take-home message:**
  - Two major recent clinical trials, BR21 and IDEAL, have concluded that EGFR protein overexpression was not predictive of response to erlotinib or gefitinib therapy and that these drugs provided maximum benefit in patients with tyrosine kinase mutations.
  - However, meta-analysis of data from 5000 pulmonary adenocarcinoma patients reported in 27 studies, including both the B21 and IDEAL trial results, showed that IHC, PCR and FISH were all associated with significant response to tyrosine kinase inhibitors therapy.
  - The current study suggests that PCR when used as a single test is likely to underestimate the presence of EGFR abnormalities that may predict response to tyrosine kinase inhibitors.
  - There is a need to develop evidence-based or consensus standardization guidelines for the performance and interpretation of EGFR tests in routine clinical practice.

- **Background:**
  - Lung adenocarcinomas with micropapillary pattern (MPP) are associated with frequent nodal metastasis.

- **Purpose:**
  - To investigate the relationship between “small cluster invasion” (SCI) and lymph node metastasis.

- **Methods:**
  - “SCI was defined as markedly resolved acinar–papillary tumor structures with single or small clusters of carcinoma cells invading stroma within fibrotic foci.”
  - 146 cases of pT1 lung adenocarcinomas were analyzed with reference to the presence of MPP, SCI, and lymphatic involvement (D2-40).

- **Results:**
  - The MPP-positive group (88/146 cases) was associated with significantly more frequent nodal metastasis and significantly worse survival.
  - SCI was significantly more frequent in the MPP-positive group (71/88 cases) than MPP-negative group (10/58 cases) and was associated with lymphatic involvement (p<0.0001) and nodal metastasis (p=0.0073).
  - The SCI positive group showed significantly worse survival (5-year survival, 70%) than the SCI-negative group (91%, p=0.0017).

- **Take-home message:**
  - SCI could link MPP to nodal metastasis (MPP → SCI → lymphatic invasion → lymph node metastasis)
II. ARTICLES FOR NOTATION ONLY
Neoplastic diseases

- **Background:**
  - Loss of tight junction function may lead to loss of cell–cell adhesion and promote tumor metastasis.
  - Claudin-1 (CLDN1), a key component of tight junctions, is downregulated in lung adenocarcinoma, but its role in cancer progression is unknown.

- **Purpose:**
  - To investigate the clinical significance of CLDN1 expression in patients with lung adenocarcinoma and its role in cancer invasion and metastasis.

- **Methods:**
  - CLDN1 mRNA expression was analyzed by real-time qRT-PCR in 64 pulmonary adenocarcinomas.
  - CLDN1 protein expression was analyzed by immunohistochemistry in 67 pulmonary adenocarcinomas.
  - CLDN1 functions in cancer cell migration, invasion, and metastatic colonization were studied by overexpression and knockdown of CLDN1 in the CL1-5 human adenocarcinoma cell line.

- **Results:**
  - Low CLDN1 mRNA expression had shorter overall survival (P<0.027, log-rank test) in 64 patients with lung adenocarcinoma.
  - Similarly, low CLDN1 protein expression had shorter overall survival (P<0.024, log-rank test) in an independent cohort of 67 patients with lung adenocarcinoma.
  - Overexpression of CLDN1 in the CL1-5 cell line inhibited cancer cell dissociation, migration, invasion, and metastasis. Knockdown of CLDN1 in the CL1-5 cell line increased cancer cell invasion and metastasis.

- **Take-home message:**
  - CLDN1 inhibits lung adenocarcinoma cells from dissociating from each other and regulates cancer cell migration, invasion and metastasis.
  - CLDN1 may be an invasion/metastasis suppressor as well as a useful prognostic biomarker and potential therapeutic target.

- **Background:**
  o Studies on a variety of cell lines have shown that p120-catenin can directly regulate the stability of E-cadherin complexes and control the activity of small GTPases to influence cell adhesion.

- **Purpose:**
  o To explore the correlation between p120-catenin, E-cadherin, and small GTPases in human lung cancer.

- **Method:**
  o The authors examined the expression patterns of p120-catenin, E-cadherin, RhoA, Cdc42, and Rac1, and their prognostic significance in 138 patients with non-small cell lung cancer (NSCLC).
  o They also used an in vitro model to evaluate the expression of these proteins and to determine whether protein expression correlated with the invasive capacity of lung cancer cell lines.

- **Results:**
  o Normal bronchial epithelium showed strong membrane expression of p120-catenin and E-cadherin
  o Lung cancer tissues had reduced membrane expression and ectopic cytoplasmic expression of p120-catenin and E-cadherin.
  o Expression of RhoA, Cdc42, and Rac1 was also found to be higher in tumor tissue than in normal lung tissue.
  o A correlation between abnormal p120-catenin, E-cadherin expression, and overexpression of specific small GTPases was also associated with poor differentiation, high TNM stage, and lymph node metastasis in NSCLC patients.
  o Consistent with the in vivo data, abnormal expression of p120-catenin and E-cadherin with overexpression of specific small GTPases were associated with the high metastatic capacity of BE1 cells.

- **Take-home message:**
  o The authors conclude that abnormal p120-catenin expression correlates with abnormal E-cadherin expression and specific small GTPase overexpression in NSCLC.
  o The study would be interesting, but the title does not make much sense.

- **Background:**
  - There is a marked survival advantage for patients with non-small cell lung cancer (NSCLC) expressing high numbers of macrophages in their tumor islets.

- **Purpose:**
  - To determine the immunological phenotype of NSCLC-associated macrophages.

- **Methods:**
  - CD68+ macrophages expressing markers of a cytotoxic M1 phenotype or a noncytotoxic M2 phenotype were identified in the islets and stroma of surgically resected tumors from 20 patients with extended survival (median 92.7 months) and 20 with poor survival (median 7.7 months), using immunohistochemistry.

- **Results:**
  - The islet density of both M1 and M2 macrophages was markedly increased in extended compared with poor survival patients.
  - In the extended survival group, M1 islet density was significantly increased compared with M2 density.
  - The 5-yr survival for patients with above and below median expression of M1 macrophages in the islets was >75 and <5%, respectively.

- **Take-home message:**
  - Macrophages infiltrating the tumor islets in non-small cell lung cancer were predominantly of the M1 phenotype in patients with extended survival.
  - The survival advantage conferred by islet macrophage infiltration may be related to their cytotoxic antitumor activity.

- **Background:**
  - Patients with N2 nonsmall cell lung cancer (N2-NSCLC) represent heterogeneous groups.
  - Survivin is a member of the inhibitor of apoptosis family.

- **Purpose:**
  - To stratify N2-NSCLC patients into subgroups based on survivin expression.

- **Methods:**
  - Survivin expression in 78 surgically resected primary pathological N2-NSCLC tumors was evaluated using immunohistochemistry.
  - Relationships of survivin expression to overall survival, clinical features and expression of six cell cycle-related proteins (pRb, cyclin D1, p16INK4A, p53, p21Waf1 and Ki-67) were analyzed.

- **Results:**
  - Nuclear survivin and the number of mediastinal lymph node (LN) stations were independent prognostic factors.
  - The patient group with combined negative survivin/single mediastinal LN station was the most favorable prognostic group, and was related to the clinical nodal factor.
  - Patients with negative survivin/low Ki-67 labeling indices had the best survival, especially in nonsquamous histopathology.

- **Take-home message:**
  - The authors conclude that nuclear survivin is strongly related to lymph node metastasis and proliferative potentials in pathological N2 nonsmall cell lung cancer patients.

Review

- D2-40 is a commercially available monoclonal antibody directed against human podoplanin, a transmembrane mucoprotein that is expressed in lymphatic endothelial cells.
- D2-40 immunoreactivity in neoplasms:
  - Vascular neoplasms:
    - Lymphangioma
    - Kaposi sarcoma
    - Hemangioendothelioma
  - Nonvascular neoplasms:
    - Epithelioid mesothelioma
    - Seminoma
    - Hemangioblastoma
    - Primary adrenal cortical tumors
    - Schwannomas
    - Adnexal tumors of the skin

Review

- The discovery of mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) accelerated the research of molecular-targeted therapy by EGFR-tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib.
- About 90% of EGFR mutations are clustered in exons 19 (deletion) and 21 (point mutation at codon 858) and patients with these mutations have great response to EGFR-TKIs.
- However, tumors that initially respond to EGFR-TKIs almost inevitably become resistant later and T790M secondary mutation in the EGFR gene and MET amplification are reported to account for the mechanism of this acquired resistance.
Non-neoplastic diseases

- **Background:**
  - Leptin is a pleiotropic cytokine important in the regulation of immune responses via its functional receptor Ob-Rb.

- **Purpose:**
  - This study was undertaken to test the hypothesis that severe COPD is associated with increased leptin expression in epithelial cells.

- **Methods:**
  - Immunohistochemistry for leptin was performed on peripheral lung specimens from
    - 20 patients with COPD,
    - 14 asymptomatic ex-smokers and
    - 13 never smokers.
  - Leptin and Ob-Rb mRNA expression were determined by rtPCR in cultured primary bronchial epithelial cells and primary type II pneumocytes.
  - NCI-H292 and A549 cell lines were used to study functional activation of leptin signaling.

- **Results:**
  - Leptin immunoreactivity in lung tissue was observed in bronchial epithelial cells, type II pneumocytes, macrophages (tissue/alveolar) and interstitial lymphocytic infiltrates.
  - rtPCR analysis confirmed pulmonary leptin and Ob-Rb mRNA expression in primary bronchial epithelial cells and pneumocytes.
  - Leptin-expressing bronchial epithelial cells and alveolar macrophages were markedly higher in patients with severe COPD and ex-smokers than in never smokers (p,0.02).
  - Exposure of cultured primary bronchial epithelial cells to smoke resulted in increased expression of both leptin and Ob-Rb (p,0.05).
  - Leptin induced phosphorylation of STAT3 in both NCI-H292 and A549 cells.

- **Take-home message:**
  - Leptin expression is increased in bronchial epithelial cells and alveolar macrophages of ex-smokers with or without severe COPD compared with never smokers.
  - A functional leptin signaling pathway is present in lung epithelial cells.

- **Purpose:**
  - To investigate matrix metalloproteinases (MMP-1, -2 and -9) and tissue inhibitors of metalloproteinases (TIMP-1 and -2) in tuberculous effusions.
  - To correlate the results with clinical and histopathological features.

- **Methods:**
  - Clinical data, routine blood tests, and pleural fluid/biopsy material were obtained from 89 patients presenting with pleural effusions in a TB-endemic area.
  - MMP-1, -2 and -9 were measured by zymography or western blot, and TIMP-1 and -2 by ELISA.
  - Pleural biopsies were examined microscopically, cultured for acid–alcohol fast bacilli and immunostained for MMP-9.

- **Results:**
  - Tuberculous pleural effusions contained the highest concentrations of MMP-9 compared with malignant effusions or heart failure transudates.
  - MMP-9 concentrations were highest in effusions from patients with granulomatous biopsies.
  - MMP-1 and -2 were not upregulated in tuberculous pleural fluid.
  - The ratio of MMP-9:TIMP-1 was significantly higher in TB effusions.

- **Take-home message:**
  - MMP-9 may play a specific role in inflammatory responses in tuberculous pleural disease.

Case study

- The study reports a case of biopsy-proven pulmonary veno-occlusive disease as a cause of severe pulmonary hypertension in a patient suffering from a chronic myeloproliferative disorder.
- The pulmonary disease responded favorably to treatment with defibrotide, a pro-fibrinolytic medication used in hepatic veno-occlusive disease.