PULMONARY PATHOLOGY JOURNAL CLUB
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I. DISCUSSION ARTICLES

Purpose:
New adenocarcinoma classification is necessary as a result of advances in oncology, molecular biology, pathology, radiology, and surgery. This new classification provides uniform terminology and diagnostic criteria (especially for BAC), the overall approach to small nonresection cancer specimens, and multidisciplinary management of tissue for molecular and IHC studies.

Methods:
An international panel of experts performed a systematic review of the literature and held a series of meetings.

Results:
Recommendations:
1. Discontinue use of term “BAC.” Replace it with the most appropriate of the following:
   a. Adenocarcinoma in situ (AIS), which can be nonmucinous and rarely mucinous
   b. Minimally invasive adenocarcinoma (MIA), which can be nonmucinous and rarely mucinous
   c. Lepidic predominant adenocarcinoma (nonmucinous)
   d. Adenocarcinoma, predominantly invasive with some nonmucinous lepidic component
   e. Invasive mucinous adenocarcinoma
2. For <3 cm solitary adenocarcinomas with pure lepidic growth, use the term AIS.
   a. These patients have 100% disease-specific survival if the lesion is completely resected.
   b. Most cases are nonmucinous.
3. For <3 cm solitary adenocarcinomas with predominant lepidic growth and small foci of invasion measuring <0.5 cm, use term MIA.
   a. These patients have near 100% disease-specific survival, if completely resected.
   b. Most cases are nonmucinous.
   c. The invasive component to be measured is defined as follows:
      i. Histologic subtypes other than lepidic OR
      ii. Tumor cells infiltrating myofibroblastic stroma
   d. MIA is excluded if the tumor 1) invades lymphatics, blood vessels, or pleura or 2) contains tumor necrosis.
   e. If multiple microinvasive areas are present in one tumor, the size of the largest area should be measured in the largest dimension. The size of invasion is not the summation of all such foci, if there is more than one.
4. For invasive adenocarcinomas, comprehensive histologic subtyping should be used to assess histologic patterns semiquantitatively in 5% increments, choosing a single predominant pattern. Individual tumors are classified according to the predominant pattern and the percentages of the subtypes are also reported.
   a. Provides a way to compare the histology of multiple adenocarcinomas.
5. Comprehensive histologic subtyping may help distinguish if multiple lung adenocarcinomas are metastases or separate primaries.
6. Use term “lepidic predominant adenocarcinoma” (LPA) for nonmucinous ADCs previously classified as mixed subtype where the predominant subtype consists of “former nonmucinous BAC.” Also, discontinue use of term “mixed subtype.”
   a. Lepidic growth is associated with favorable survival.
7. Use “micropapillary predominant adenocarcinoma” as a major histologic subtype in patients with early-stage ADC.
   a. Poor prognosis.
   b. Overrepresentation of micropapillary pattern in metastases compared with primary tumors.
8. Formerly mucinous BAC should be separated from former nonmucinous BAC. Mucinous BAC $\rightarrow$ mucinous AIS, mucinous MIA, or for overtly invasive tumors “invasive mucinous ADC.”
   a. Mucinous associated with KRAS mutation, nonmucinous more likely to have EGFR mutation.
9. For small biopsies and cytology, NSCLC should be further classified whenever possible.

**Conclusions:**
- This new classification has implications for TNM staging.
- AIS would be classified as Tis.
- MIA would be classified as T1mi.
- In early stage tumors, tumor size (T) may be adjusted from total tumor size to only size of invasive component.
- For multiple lung ADCs, comprehensive histologic subtyping can help distinguishing intrapulmonary metastasis versus multiple primaries.
Wei et al. Which is the better prognostic factor for resected non-small cell lung cancer – the number of metastatic lymph nodes or the currently used nodal stage classification? J Thorac Oncol. 2011;6:310-318.

**Background:**
Retrospective study was conducted to evaluate prognostic significance of the number of metastatic lymph nodes (nN) in resected NSCLC in comparison with currently used location-based staging system (pN).

pN is not ideal because there is heterogeneity of pN1 and pN2 with regard to prognosis and differences in labeling between surgeons.

**Methods:**
1659 patients who underwent potentially curative resection for NSCLC from 2000-2006 were included. Association between nN and survival compared with association between pN and survival.

**Results:**
Patients divided into four categories:

<table>
<thead>
<tr>
<th>Classification</th>
<th># node mets</th>
<th>5 year survival</th>
</tr>
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<tbody>
<tr>
<td>nN0</td>
<td>0</td>
<td>89.2</td>
</tr>
<tr>
<td>nN1</td>
<td>1-2</td>
<td>65.1</td>
</tr>
<tr>
<td>nN2</td>
<td>3-6</td>
<td>42.1</td>
</tr>
<tr>
<td>nN3</td>
<td>7</td>
<td>22.4</td>
</tr>
</tbody>
</table>

P < 0.001

Multivariate analysis showed that the nN category was an independent prognostic factor.

Difference in overall survival between pN1 and pN2 was not significant (55.4 vs 47.8, p = 0.245).

nN does not have as strong a discriminative ability in pN1 as in pN2 patients (lymph node fragments – overestimating # of positive lymph nodes?)

**Conclusions:** nN is a better prognostic determinant than location-based pN.

Previous studies have shown excellent agreement between pN and nN, stepwise deterioration in prognosis with increase in number of positive nodes, and that nN is an independent prognostic factor. N1 lymph nodes are more likely to be removed in fragments than N2 lymph nodes because of adhesion to bronchus and lung tissues.

For metastasis in the same number of lymph nodes, the location (pN1 or pN2) is not important for postoperative survival.

Limitations: Lymph node fragments – may overestimate number of metastatic nodes.
Sufficient number of retrieved lymph nodes is necessary – no standard.
Number of positive lymph nodes difficult to assess in nonsurgical patients.
Optimal category definition for the number of metastatic lymph nodes needs to be further explored.

**Background:** Aim was to assess significance of microscopic vascular invasion (MVI) in patients with early-stage NSCLC and analyze effect of combination of MVI and tumor size for T-size categories T1a-T2b.

**Methods:** 1993-2008, 746 patients with pT1-pT2N0 NSCLC who received resection. MVI was defined as presence of neoplastic cells inside either blood or lymphatic vessels, and usually consisted of neoplastic cells in an organized vascular thrombosis. If any doubt whether neoplastic cells could represent artifacts, specimen underwent serial sections, and/or CD34 staining was used. Follow-up with vital status was available for all patients, with mean follow-up time of 53 months.

**Results:** MVI observed in 34% of cases. In 30% of these, IHC with CD34 was necessary for final diagnosis of MVI. MVI prevalence was significantly higher in ADC (44%) than in SCC (27%, p = 0.002). MVI was significantly associated with the presence of tumor infiltrating lymphocytes (p = 0.02), increased tumor size (p = 0.05), and adenocarcinoma subtype (p = 0.03).

In univariate survival analysis, MVI was associated with decreased survival at 5 years (65% MVI -, 55% MVI +, p = 0.001). In SCC, MVI was not associated with a significant 5 year survival difference. In ADC, MVI was significantly associated with worse 5 year survival (71% MVI -, 60% MVI +, p = 0.002).

**Conclusions:**
- MVI is frequent in resected patients with NSCLC.
- MVI correlates with tumor size, presence of tumor-infiltrating lymphocytes, and adenocarcinoma type.
- MVI is independent negative prognostic factor.
- The 2009 TNM staging system underestimates the presence of MVI.

**Background:** NSCLC with activation mutations of *EGFR* generally respond to EGFR TKIs (gefitinib or erlotinib). Miliary pulmonary metastases are most commonly observed in patients with thyroid carcinoma but can be seen in patients with lung cancer. An association between multiple pulmonary metastases, particularly military metastases, and response to gefitinib therapy in patients with NSCLC has been reported.

**Methods:** Retrospective investigation of the association of diffuse, random pulmonary metastases in patients with 163 patients with unresectable, advanced lung adenocarcinoma. Diffuse, random pulmonary metastases were defined as multiple nodules distributed diffusely and randomly throughout the lungs. CT scans were analyzed by 2 reviewers.

**Results:** Of 163 patients, 55 have pulmonary metastases, and *EGFR* mutations were detected in 22 of 55 patients. Miliary metastases were identified in 11/22 patients with *EGFR* mutations and 4/33 patients with wild-type *EGFR* (p = 0.0043). Multivariate analysis showed that *EGFR* mutations were associated independently with a smoking history of <10 pack years and with military metastases.

**Conclusion:** Patients who had lung ADC with *EGFR* mutations tended to develop military metastases. Miliary metastases were much less common in lung ADCs with wild-type *EGFR*. *EGFR* mutations that accompany cancer, including NSCLC and thyroid carcinoma, tend to develop angiogenic metastases.

**Background:**
Increased cell components are spilled into bloodstream during carcinogenesis. Free human telomerase reverse transcriptase (hTERT) serves as surrogate of circulating DNA. Levels are higher in patients with lung cancer than in healthy controls.

**Purpose:**
Prospectively study the association between hTERT in plasma and clinical variables and survival in a large-scale NSCLC study.

**Methods:**
446 patients with stages IIIB and IV NSCLC with median follow up of 9.7 months were analyzed. Blood samples were collected before start of therapy.

**Results:**
Patients with less than the median value of hTERT had a median time to progression of 6.3 months compared with 4.9 months for higher levels of hTERT (p = 0.001). Overall survival was significantly higher (10.9 versus 9.3 months) at lower hTERT levels (p = 0.012).

**Conclusions:**
In advanced NSCLC, high pretreatment circulating hTERT level is an independent poor prognostic marker for time to progression and overall survival.

**Background:** Zeb1 and twist regulate expression of genes which take part in epitheliomesenchymal transition (EMT). Claudins are tight junctional proteins regulating polarity and paracellular permeability of epithelial cells. The spread of malignant cells is believed to be due to EMT, a process where epithelial carcinoma cells gain fibroblastic properties and are therefore able to invade surrounding structures and metastasize. In EMT there is down-regulation of epithelial adhesion genes and up-regulation of mesenchymal fibroblast-type genes.

Zeb-1 (zing-finger E-box-binding homeobox 1) induces EMT and down-regulates E-cadherin in epithelial cells. Twist is a helix-loop-helix transcriptional factor which promotes EMT and down-regulates E-cadherin.

**Methods:** Zeb1, twist, and claudins 1-5 and 7 expression by IHC in 289 primary and 54 metastatic lung tumors.

**Results:** Metastatic tumors more frequently expressed zeb1 and twist than primary tumors (both p < 0.001). Zeb 1 and twist are strongly inversely associated with claudin expression.

In primary lung tumors, expression of claudins 1-5 and 7 did not associate with survival. In metastatic tumors, strong expression of claudin 5 associated with worse survival.

**Conclusions:**

- Zeb1 and twist are often expression in stromal compartment of lung tumors but are rare in epithelial component.
- Metastatic tumors to the lung show a significantly higher expression of zeb1 and twist in the epithelial cell compartment than primary lung tumors.
- Zeb1 and twist expression is inversely associated with some claudins.
Cappabianca et al. Preliminary study on the correlation between grading and histology of solitary pulmonary nodules and contrast enhancement and \(^{18}\text{F}\)fluorodeoxyglucose standardized uptake value after evaluation by dynamic multiphase CT and PET/CT. J Clin Pathol. 2011;64:114-119.

**Background:** Overall incidence of malignancy in solitary pulmonary nodules is 30-40%. Aim of study was to evaluate whether the histology and grading of solitary pulmonary nodules (SPN) correlated with the results of dynamic multiphase multidetector CT (MDCT) and the \(^{18}\text{F}\)fluorodeoxyglucose standardized uptake value (SUV) in 30 patients.

**Methods:** Retrospective evaluation of 270 chest x-rays of patients with incidentally detected SPNs. On MDCT and PET/CT images, two experts measured the density of nodules in all perfusion phases and the SUV.

**Results:**
- 30 of the SPNs were malignant. Six were classified as G1 (grade 1), 15 as G2, and nine as G3.
- A highly negative correlation was found in grade 3 SPNs between net enhancement and the corresponding diameters.
  - Net decrease in perfusion despite an increase in dimension.

**Conclusions:**
Median NE and SUV were highest in G2 lesions. There is a net decrease in perfusion in G3 SPNs. Previous reports have suggested a decrease in microvessel density with increasing tumor grade.

**Limitations:** Small number of patients, no description of histologic grading.

**Case report:** Sugar tumors are uncommon tumors belonging to the family of perivascular epithelioid cell tumors (PEComas). The majority act in a benign fashion. No previous pigmented variants have been described. This is the first known case of a pigmented CCST of the lung with malignant histological features.

Neoplastic cells were positive for S100, muscle specific actin, HMB45 and vimentin. Criteria have been proposed for PEComas of soft tissue and gynecologic origin, but do not exist for pulmonary PEComas due to their extreme rarity.

Differential diagnosis – melanoma? No previous history, Indonesian Chinese origin, clear cell features, immunohistochemical expression of muscle markers argue against melanoma.

Despite significant atypia, patient did not manifest any tumor recurrence or metastasis during a 10 year follow-up.
Background: Sarcoidosis is a multisystemic granulomatous disease of unknown etiology characterized by compartmentalization of CD4+ T helper 1 lymphocytes (Th1). A complex network of cytokines and chemokines is involved in the pathogenesis. Th17 is a recently described CD4+ effector T cell population. Hypothesis: Th17 cells are involved in pathogenesis of sarcoidosis.

Methods: Peripheral and pulmonary Th17 cells were evaluated by flow cytometry, real-time PCR, IHC and functional analysis in 40 patients with sarcoidosis and 10 control subjects.

Results: In peripheral blood, mean percentage of IL-17+ T cells in the CD4+ subset was 4.72 in patients with active sarcoidosis, versus 0.63% IL-17+/CD4+ T cell subset in patients with inactive disease (p = 0.001) and 0.67% in controls (p = 0.001).

Freshly isolated alveolar macrophages in BAL show significantly more intracellular IL-17 expression than patients with inactive sarcoidosis and controls.

IHC analysis showed IL-17 by sarcoid lung T cells infiltrating surgical lung biopsies in four patients with active sarcoidosis.

Conclusions: Th17 cells infiltrate sarcoid lung, localizing around and inside the granuloma. Evidence that Th17 cells are increased in lung and peripheral blood of patients with active sarcoidosis supports multisystem nature of disease.

**Background:**
COPD is a chronic inflammatory condition, which is associated with the development of ectopic lymphoid follicles. COPD progression is associated with increased number of bronchioles containing lymphoid follicles. Purpose was to examine prevalence, localization, vascularization, addressin expression and inflammatory cell densities of lymphoid follicles in lung tissue of moderate and very severe COPD patients compared to nonsmokers and smokers without COPD.

**Methods:**
59 subjects who underwent lung resection for peripheral lung tumors of transplant for severe COPD. Subjects were classified into four clinical groups according to smoking habits and COPD severity.

**Results:**
- COPD patients had a significantly higher density of CD57+ cells than did nonsmokers and smokers without COPD.
- No significant differences in the densities of the other cell types were found.

**Conclusions:**
- There is a significant and specific increase in the follicular density of CD57+ cells in patients with COPD.
- Previous evidence has suggested that CD57 is a marker of lung inflammation or general immune dysfunction.
- CD57+ cells could play a key role in COPD pathogenesis.
Background:
Incidence, risk factors and outcomes of acute exacerbation (AE) of IPF are unknown. Clinically similar rapid deterioration (RD) can be caused by other conditions.

Methods:
Retrospective review of 461 patients diagnosed as having IPF according to the ATS/ERS consensus classification. RD was defined as a <30 day worsening of dyspnea requiring hospitalization and the presence of newly developed radiologic abnormalities. AE was defined as a sudden worsening of dyspnea within 30 days with new bilateral lung infiltration in patients with known IPF. BALs were performed to rule out infection. Patients diagnosed with IPF at the time of AE were excluded.

Results and Conclusion:
- Median follow-up was 22.9 months. 1- and 3-year incidences of first event AE were 11.6 and 18.2%. 1- and 3- year incidences of total RD were 23.0 and 35.4%. AE was the most frequent case of RD, followed by infection.
- Significant risk factors for AE were low FVC and never smoking. Most had no identifiable precipitating factors. The immediate outcome of AE was very poor (median survival 2.2 months). AE had significant poor impact on overall survival, along with old age, low FVC, and steroid treatment.
Background:
Vitamin D deficiency has been implicated in the development of autoimmune diseases. Aim of this study was to evaluate the prevalence of vitamin D deficiency in a cohort of patients with ILD.

Hypothesis: Vitamin D deficiency would be associated with an underlying connective tissue disease (CTD) and reduced lung function.

Methods:
118 patients with ILD (67 with CTD-ILD and 51 with other ILD) were included. Regression analysis evaluated associations between 25-hydroxyvitamin D and other variables.

Results:
- CTD-ILD patients were more likely to be women, younger, and have taken corticosteroids.
- 58% of the cohort were insufficient in vitamin D.
- The mean vitamin D level was significantly lower in those with CTD-ILD as opposed to other forms of ILD (20.8 vs. 33.1 ng/mL, p < 0.0001).
  - Not explained by corticosteroid usage.
- Among those subjects with CTD-ILD, reduced vitamin D levels were strongly associated with reduced lung function.

Conclusions:
Other studies have shown vitamin D is implicated in the development of lung disease. In patients with COPD, there is an association between FEV1 and vitamin D levels even when adjusted for activity level. The pathogenic relationship has yet to be established. Prospective controlled interventional studies are needed to determined if vitamin D supplementation can ameliorate symptoms and improve outcomes in patients with CTD-ILD.

Limitation – All done in Cincinnati. Possible difference in warmer/more sun exposed climates?

**Background:**
Microcrystalline cellulose (MCC) is extensively used as a filler material in pharmaceutical tablets.

**Methods:**
FFPE lung tissues containing birefringent crystals were evaluated with modified Russell Movat pentachrome stain (MMPS).

**Results:**
MMPS stained the MCC bright yellow, talc stained light greenish-blue, oxalate stained sea-green, and crospovidone stained yellow to dark green.