PULMONARY PATHOLOGY JOURNAL CLUB
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Table of Contents

Discussion articles
Page 2  Kadota et al. Tumor Spread through Air Spaces is an Important Pattern of Invasion and Impacts the Frequency and Location of Recurrences after Limited Resection for Small Stage I Lung Adenocarcinomas. J Thorac Oncol 2015;10: 806-14.


Articles for notation


Augustin et al. Receptor for hyaluronic acid-mediated motility (RHAMM, CD168) expression is prognostically important in both nodal negative and nodal positive large cell lung cancer. J Clin Pathol 2015;68: 368-73.


Discussion articles
Kadota et al. Tumor Spread through Air Spaces is an Important Pattern of Invasion and Impacts the Frequency and Location of Recurrences after Limited Resection for Small Stage I Lung Adenocarcinomas. J Thorac Oncol 2015;10: 806-14.

Purpose: To explore the significance of tumor spread through air spaces (STAS), referred to historically as aerogenous spread, in resected early stage lung adenocarcinomas.

Methods: Retrospective review of small (≤ 2 cm) stage I lung adenocarcinomas resected between 1995 and 2006.

- Excluded patients with any of the following features: neoadjuvant therapy, multiple nodules, positive margins, other lung cancer surgeries within the last 2 years, other disease progression, or no slides available for review.
- n = 411
- updated follow-up through March 2014
- recurrences defined as local (same lobe and/or surgical margin), regional (ipsilateral lobe and/or mediastinal lymph nodes) and/or distant (contralateral lung and/or mediastinal lymph nodes, ipsilateral supraclavicular lymph node, or outside the hemithorax of origin).
- STAS = tumor cells within air spaces beyond the edge of the tumor comprising micropapillary, solid nests, and/or single cells
- distance from furthest STAS to tumor edge measured with a ruler

Results:

<table>
<thead>
<tr>
<th>Outcome (CIR)</th>
<th>Limited resection (120)*</th>
<th>Lobectomy (291)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>locoregional</td>
<td>28 (23%)</td>
<td>30 (10%)</td>
</tr>
<tr>
<td>distant</td>
<td>14 (12%)</td>
<td>6 (2%)</td>
</tr>
<tr>
<td>14 (12%)</td>
<td></td>
<td>24 (8%)</td>
</tr>
<tr>
<td>Dead of any cause w/out recurrence</td>
<td>37 (31%)</td>
<td>62 (21%)</td>
</tr>
<tr>
<td>Variable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (&lt;65; ≥ 65)</td>
<td>0.046</td>
<td>0.022</td>
</tr>
<tr>
<td>Tumor size (≤ 1 cm; &gt; 1 cm)</td>
<td>0.004</td>
<td>0.64</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>Histologic subtype</td>
<td>0.22</td>
<td>0.006</td>
</tr>
</tbody>
</table>

*68 (57%) underwent lymphadenectomy or sampling

- Predictable risk factors correlated with outcome but the differences were variable; histologic subtype (ie, micropapillary and solid) mattered only in lobectomy patients
- STAS in 155 (38%) of cases: micropapillary (94) > solid (53) > single cells (8)
  - lymphatic (42% vs 27%) and vascular (28% vs 19%) invasion, micropapillary (83% vs 30%) and solid (46% vs 30%) growth patterns more common in STAS-positive tumors
  - recurrence more common in STAS-positive tumors in limited resection patients (5 yr CIR 42.6% vs 10.9%); STAS the only risk factor for recurrence in multivariate analysis (P = 0.014).
  - minimal impact (P = 0.045) on 5-year OS rates in limited resection patients

Take-home message: STAS may or may not be “invasion” in the traditional sense, is predictably affiliated with a micropapillary growth pattern (in fact, it is kinda the same thing), and therefore not surprisingly predicts for recurrence risk in patients undergoing limited resection. Importance beyond that is nil.

**Purpose:** To investigate the prognostic significance and survival outcomes in patients with a minimal proportion of micropapillary pattern (<5% total tumor) adenocarcinoma, regardless of predominant subtype.

**Methods:** Retrospective review of surgically resected lung adenocarcinomas between September 2003 and August 2011
- \( n = 525 \)
- Classified according to IASLC/ATS/ERS lung adenocarcinoma classification guidelines, subtyping at five percent increments, and identifying the predominant pattern
- Assessed for presence of micropapillary pattern less than five percent, and quantified at one percent intervals (<1% considered absent)
- Classified into three subgroups based upon presence and proportion of micropapillary pattern in entire tumor; (1) Micropapillary pattern \( \geq 5\% \) \((n = 114)\), (2) Micropapillary pattern <5% \((n = 115)\), (3) Absent \((n = 296)\)
- Assessed overall survival (OS) and disease-free survival (DFS) using clinical and histologic characteristics

**Results:**
- Frequency of EGFR mutation increased as extent of micropapillary pattern increased \((65\% \text{ in } 0 \text{ group}; 82\% \text{ in } <5\% \text{ group}; 100\% \text{ in } \geq 5\% \text{ group})\)
- OS significantly better in patients without micropapillary pattern than those with <5% \((P = 0.034)\) or \(\geq 5\% \ (P = 0.034)\); but, no significant difference between <5% and \(\geq 5\% \) groups \((P = 0.893)\)
- DFS significantly better in absent and <5% groups compared to \(\geq 5\% \) group \((P < 0.001 \text{ and } P = 0.039, \text{ respectively})\)
- Prognosis (DFS, \(P < 0.001\); OS, \(P = 0.453\)) based upon predominant subtype: lepidic > acinar > papillary > solid > micropapillary
- Age \((P = 0.005)\) and N status \((P = 0.005)\) independent prognostic factors influencing OS (inverse relationship)

**Take-home message:** Even a minute proportion of micropapillary pattern (>1%) of adenocarcinoma may warrant mentioning in reporting, since its mere presence has been observed to have significant prognostic implications, particularly overall survival.

**Purpose:** To elaborate on the technical performance of multiplex molecular assays used to support a multi-institutional program to interrogate the mutational profile of high stage lung adenocarcinomas (ACA). Previously reported results demonstrated driver mutations in 64% of 733 tumors for which full genotyping (*ie*, 10 genes) was performed (Kris et al. JAMA 2014; 311: 1998).

**Methods:**
- 14 clinical sites; 6 testing sites accounted for >90% of testing (Univ of CO, MSKCC, MGH, BWH, Emory, NCI); 1 additional testing site accounted for 1% of the results
- inclusion criteria: stage IV or recurrent lung ACA; SWOG performance status 0, 1, 2; expected survival > 6 mos; adequate tissue (inadequate tissue accounted for 65% of 440 ineligible patients)
- 1007 ≥ 1 assay; 989 ≥ 1 small mutations (single nucleotide variants, indel); 926 ALK FISH; 833 MET FISH; 733 10-marker panel
- complete panel for small mutations: 4 indels and 93 point mutations in 8 genes (*AKT1, BRAF, EGFR, ERBB2, KRAS, MAP2K1, NRAS, PIK3CA*)
- assays variable and included SNapShot (Life Technologies), Sequenom MassARRAY (Sequenom), PCR-sizing, Sanger ± peptide nucleic acid (PNA) clamps
- interinstitutional validation cohort (18 cell lines, patient FFPE samples: 9 DNA, 9 ALK & 2 MET FISH)
- independent proficiency testing protocol (5 DNA samples, 2 ALK & 2 MET FISH)

**Results:**
- There were no significant differences between specimen types (*ie*, biopsy, cytology, surgical) in mean call rates or detection rates for either small mutation genotyping or ALK FISH
- site with highest mutational testing volume (470) showed failure rates significantly lower (*P*<0.001) for surgical specimens (5%) compared to biopsies (26%) and cytologies (35%) with an overall fail rate of 15%; these rates an underestimate given selection criteria for study entry
- 8/9 proficiency samples correctly scored; 1/9 gave unexpected results (no consensus among sites)
- 27 (2.7%) of 1007 samples with any testing had multiple driver mutations
  - 14 with 2 small mutations
  - 9 MET amplification + small mutation (4-KRAS, 2-EGFR, 1-ERBB2) or ALK rearrangement (2);
  - 4 ALK rearrangement* + small mutation (3-EGFR, 1-BRAF)
  *orthogonal/repeat testing failed to confirm in 2; insufficient tissue for repeat testing in 1
- multiple oncogenic drivers (*EGFR, KRAS, ERBB2 ALK*) showed significant associations with demographic (gender, race, smoking) and prognostic (stage, adrenal and bone mets) factors
- no significant association between histology and mutations in resected tumors (% of total cohort); trend toward ↑*EGFR* in acinar (25%) compared to solid (14%) subtypes

**Take-home message:** When it comes to multiplex genotyping assays, the specimen quality and quantity matters way more than the platform itself (*ie*, the age old maxim, *garbage in, garbage out*, still applies!). Increasingly important that pathologists be responsible stewards of diagnostic tissues to preserve the interests of patients with advanced disease (*ie*, doing large IHC panels without a clearly identified question requiring resolution should not stand).

<table>
<thead>
<tr>
<th></th>
<th>Any testing (1007)</th>
<th>Full testing (733)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No driver mutation</td>
<td>38%</td>
<td>36%</td>
</tr>
<tr>
<td>KRAS</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>EGFR</td>
<td>22%</td>
<td>23%</td>
</tr>
<tr>
<td>ALK</td>
<td>8.5%</td>
<td>7.9%</td>
</tr>
<tr>
<td>all others</td>
<td>&lt;3% each</td>
<td>&lt;3% each</td>
</tr>
<tr>
<td>&gt;1 gene</td>
<td>3%</td>
<td>3%</td>
</tr>
</tbody>
</table>

**Purpose**: To identify histological features that predict aggressive behavior in type A and AB thymomas.

**Methods**:
- retrospective review of resected type A and AB thymomas drawn from the files of the Royal Brompton Hospital (1992-2011) and the Institute of Pathology, University Medical Centre Mannheim, University of Heidelberg (1986-2011); consultation cases were included if slides available
- 12 cases excluded (reclassified on review, no staging information available)
- 121 study cases identified (68 type A, 53 type AB); med 4 slides/case (range 1-29)
- no relationship between # slides/case and rates of atypia, mitoses, necrosis
- insufficient follow-up for disease-specific survival analysis
- graded for, a) nuclear pleomorphism (1-3), scoring both highest and predominant grade, b) mitotic activity (5 x 10 HPFs) expressed as mean/median mits/10 HPF (2 mm²), and c) necrosis (present/absent)

**Results**:

<table>
<thead>
<tr>
<th></th>
<th>Males/Females</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Type A (68)</td>
<td>34/32</td>
<td>36 (53%)</td>
</tr>
<tr>
<td>Type AB (53)</td>
<td>21/32</td>
<td>36 (68%)</td>
</tr>
<tr>
<td>ALL (121)</td>
<td>55/64</td>
<td>72 (60%)</td>
</tr>
</tbody>
</table>

| highest median mitotic count (MMC) | 16 | 20 | 3 | 1 |
| % of cases with MMC > 10          | 4  | 2  | 0 | 0 |
| Predominant grade                |    |    |   |   |
| grade                            | 1  | 83%| 76%| 80%| 50%|
| Necrosis present                 |    |    |   |   |
|                                 | 2  | 17%| 24%| 20%| 50%|

- there were 8 cases in which the highest nuclear grade was 3 (3, 3, 1, 1 in stages I, II, III, and IV, respectively) but none in which it was the predominant grade
- only necrosis showed a significant association with advanced stage in both univariate (OR 3.53; P 0.01) and multivariate (OR 4.36; P 0.006); neither mitotic count nor grade was significant
- no recurrences in the Brompton Hospital patients (no information included from Heidelberg cohort) and no tumor-related deaths in the 79 patients for whom mortality data was available

**Take-home message**: Odd study that mostly suffers from incomplete data and numbers too small to make statistically or biologically relevant observations. Bottom line is that there is little reason to devise histologic predictors of aggressive behavior in a group of patients for whom aggressive behavior seems almost never to occur, at least of the sort that puts life at risk. Since stage did not seem to predict either recurrence or survival in this retrospective observational study, being able to predict stage seems of limited value at best. But if you wanted to engage in the exercise any way, I guess necrosis has more “value” than counting mitoses (YAY!) or assessing nuclear grade.
Articles for notation

The authors examined 32 resected lung adenocarcinomas (3AIS, 5 MIA, 19 lepidic, 3 papillary, and 2 acinar predominant) using several IHC stains focusing on an “EGFR mutation-specific antibodies.” They used two Dako antibodies directed against exon 19 deletions (clone 6B6) and point mutations in exon 21 (clone 43B2). Their study cohort was compared to 40 benign lesions in which there was pneumocyte hyperplasia (33 granulomatous inflammation, 5 “non-granulomatous chronic inflammation,” 1 organizing pneumonia, and 1 bacterial infection). They also applied IHC stains to preoperative biopsies from patients in whom resected adenocarcinomas were IHC-positive for EGFR mutations.

Twelve (38%) of 32 adenocarcinomas were IHC positive for EGFR mutations (9 point mutations and 3 deletions). Staining was characterized as diffuse (> 50%) in 9 and focal (10-50%) in 3. There was perfect concordance with the results of molecular analysis in the 12 IHC positive cases. Staining was also seen in 10 of 12 preoperative biopsies; the 2 negative cases were among the 3 with focal staining patterns in the resected tumor. Using ROC curves EGFR antibodies outperformed stains for p53 (thresholds 30% and 50%), mouse double minute 2 (MDM2) and stratifin (SFN); the highest AUC was with a combination of EGFR and p53 (30% threshold).

With a negative predictive value of only 67% and sensitivity of 38%, albeit a specificity of 100%, it is hard to imagine wanting to use this any time soon!


This just in, use of some combination of TTF1, napsin, p63, p40 and CK5/6 (they were neither standardized nor detailed in this retrospective study of 40 biopsy/cytology-surgical resection pairs) accurately subclassifies some (33/40 – 82%) but not all carcinomas that could not be otherwise subclassified on the basis of histology/cytology alone. The diagnostic changes were pretty insignificant, predictable, and based mostly on histopathology rather than reapplication of IHC stains.

<table>
<thead>
<tr>
<th>Biopsy diagnosis (#)</th>
<th>Resection diagnosis</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC, NOS (3)</td>
<td>adenocarcinoma</td>
<td>predictable outcome</td>
</tr>
<tr>
<td>squamous cell ca (2)</td>
<td>adenosquamous carcinoma</td>
<td>they were half right</td>
</tr>
<tr>
<td>adenocarcinoma (1)</td>
<td>pleomorphic carcinoma</td>
<td>TTF1/napsin positive so would be viewed as “adenocarcinoma” for purposes of triage to molecular testing/treatment</td>
</tr>
<tr>
<td>squamous cell carcinoma (1)</td>
<td>large cell carcinoma</td>
<td>This one hard to understand – biopsy p63 positive and p40 positive when tested in retrospect but resection negative. Sounds like poorly differentiated SCC to me in today’s language.</td>
</tr>
</tbody>
</table>

The authors did extensive ALK FISH analysis in biopsies (11) and excisions (15) from 20 patients who had previously tested positive for ALK rearrangements by IHC. For the excision specimens, there were 6-10 observation areas. Both biopsies and resections were analyzed by two different experienced observers. For each observation area, whether biopsies or excisions, tumors were considered positive for ALK rearrangements if ≥ 15% of at least 50 counted nuclei showed split signals.

Results:
- 5 of 9 excision specimens showed heterogeneous results with 50% (4 of 8) to 83% (5 of 6) observation areas ALK FISH positive; 4 of 9 were ALK FISH positive in all (100%) observation areas
- biopsies from 6 patients with ALK FISH positive excision specimens were positive (3), equivocal (ie, 5% to 14% positive nuclei) (2) or negative (1) by ALK FISH
- 6 patients with advanced stage disease whose biopsies were positive (4) or equivocal (2) by ALK FISH analysis underwent crizotinib treatment (note: included 1 patients from the 6 above with both biopsy and excision for comparison) and all had a clinical response.

Add this to other papers reviewed in this journal club suggesting that a combination of IHC and FISH is probably the best testing strategy for identifying patients likely to benefit from appropriately targeted therapy. Patients whose biopsies are ALK positive by IHC may benefit from crizotinib even if negative by FISH.


The authors applied an antibody directed against olfactomedin 4 (OLFM4) to FFPE tissue from 218 patients with NSCLC and showed “high expression” (high = staining index of 6-9) in 64% of patients. OLFM4 expression correlated with pathologic grade, lymph node mets, peritumoral intravascular emboli, and smoking status. Low expression (ie, staining index 0-4) in smokers correlated with shorter survival compared to patients with high expression levels, but the good news is OLFM4 expression did not emerge as an independent prognostic biomarker.

Whew! Another of countless biomarkers that is not likely to pop up on your radar screen as having value in the care of NSCLC patients.

Augustin et al. Receptor for hyaluronic acid-mediated motility (RHAMM, CD168) expression is prognostically important in both nodal negative and nodal positive large cell lung cancer. J Clin Pathol 2015;68: 368-73.

The authors performed IHC stains on tissue microarrays constructed from 383 “well characterized surgically resected NSCLCs” in order to, “assess the prognostic role of CD44v6 in the presence of its potential interaction partners CD95, OPN, RHAMM, P-gp and cleaved caspase 3 (Casp3, for quantification of apoptosis)”. OPN, RHAMM and P-gp showed statistically significant discriminatory power for disease-specific survival. RHAMM was a negative prognostic factor in patients with LCC regardless of node stage, but did not fall out as prognostic factor in multivariate analysis. P-gp expression emerged as a negative prognostic factor that was independent of stage, gender, grade, and Ki-67 index in multivariate analysis.

Not sure but don’t think these will emerge as prime time players for standard practice any time soon. My head hurts.
Bob Homer sent an invitation to PPS members to anonymously participate in an online survey intended to measure rates of agreement regarding staging of patients with multiple foci of lung carcinoma. His survey comprised 5 scenarios in which patients had multiple intrapulmonary foci of carcinoma followed by questions regarding the relationship of the foci (ie, independent primaries, mets, or uncertain). Of 80 respondents, just over half described themselves as academic pulmonary pathologists and another quarter as academic pathologists with an interest in pulmonary pathology (not sure the difference, but did make me reflect on what and who I am). Bottom line is there were variable rates of agreement that did not differ between academic and non-academic pathologists.

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>NOS</th>
<th>TTF1 discordance</th>
<th>EGFR discordance</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent primaries</td>
<td>78 (98%)</td>
<td>31 (39%)</td>
<td>67 (84%)</td>
<td>75 (94%)</td>
<td>43 (54%)</td>
<td>42 (53%)</td>
<td>48 (61%)</td>
</tr>
<tr>
<td>Intrapulmonary mets</td>
<td>0</td>
<td>11 (14%)</td>
<td>0</td>
<td>0</td>
<td>11 (14%)</td>
<td>17 (21%)</td>
<td>14 (18%)</td>
</tr>
<tr>
<td>Uncertain</td>
<td>2 (3%)</td>
<td>38 (48%)</td>
<td>13 (16%)</td>
<td>5 (6%)</td>
<td>26 (33%)</td>
<td>21 (26%)</td>
<td>17 (22%)</td>
</tr>
</tbody>
</table>

Guess the take home message is that you can do this as well as anybody – so just do it! The good news is that there is emerging evidence that it may not matter since patients with multifocal tumors do much better than AJCC stage groupings would predict.


The authors applied IHC to TMAs to demonstrate PD-L1 expression in 26 (70%) of 37 thymic carcinomas and 22 (22%) of 102 thymomas. Prevalence of PD-L1 expression varied by WHO subtype and was highest in type B (33% - 35%), lowest in type AB (8%), and intermediate in type A (22%). There was no relationship between PD-L1 expression and overall survival.

Given all of the excitement around PD-L1 these days this might come up in patients with advanced disease.


Using an Illumina NGS platform the authors performed whole exome sequencing of 6 mesothelioma (MM) cell lines and comprehensive RNA sequencing of 12 MM cell lines (including the 6 that underwent whole exome sequencing) as well as 4 clinical samples. They identified 15 novel fusion transcripts that were confirmed by PCR and included 3 fusion pairs involving protein tyrosine kinase genes. Their interest centered on large tumor suppressor 1 (LATS1)-presenilin-1 (PSEN1); LATS1-PSEN1 was demonstrated to lose tumor suppressor function in an in vitro model compared to wild type LATS1. LATS1 is a central player in the Hippo signaling pathway which regulates organ size and tissue regenerative capacity. The authors demonstrated loss of LATS1 expression and multiple (10) other key Hippo signaling proteins. Analysis of whole exome sequencing data identified alterations in 6 Hippo pathway genes. They were unable to link their findings to any demographic or prognostic significance but propose targeting the Hippo pathway in developing new therapeutic strategies.

The authors performed whole exome DNA Sanger sequencing on blood-derived DNA from a woman who died at age 50 of IPF. She and other family members suffered from a combination of pulmonary fibrosis and infertility. Lymphocyte telomere length in the proband was at the 1st age adjusted percentile, and granulocyte length was below the 10th percentile. Exome sequencing identified two mutations in the telomere binding protein TINF2. The missense mutation was the predominant variant in lung-derived DNA suggesting that it represented the germline mutation. The authors concluded that the blood-identified deletion may be acquired and had a protective effect by diminishing expression of the missense mutation. Sequencing of the proband’s father and sister who had no features of no telomere syndrome features and normal telomere lengths, failed to reveal mutations. Analysis of 40 additional probands with familial pulmonary fibrosis failed to demonstrate the TINF2 mutation.

Add TINF2 to the growing list of mutations, this one accounting for about 1% of patients with familial pulmonary fibrosis. Furthermore unexplained infertility in young adults may serve as an early sign of short telomere syndromes.