Articles for Discussion


Introduction

- In the appropriate clinical setting, the presence of a pattern suggestive of UIP on HRCT is sufficient for the diagnosis of IPF, obviating the need for a surgical lung biopsy.
- However, biopsy-proven UIP is also seen in patients without typical HRCT features of UIP.
- Patients with non-typical HRCT features of UIP may have a better prognosis than those with typical HRCT features of UIP.
- The clinical features and outcomes in those patients with radiological–pathological discordance are not well defined.

The aim of this study was to compare the clinical, radiological and histological findings in a large population of subjects enrolled during a multicentre study of idiopathic pulmonary fibrosis, with a focus on discordance between imaging and histologic diagnoses of UIP.

Methods

Patient selection
Retrospectively reviewed the HRCT and histological diagnoses in 241 subjects who had had HRCT and a surgical lung biopsy, enrolled in three studies sponsored by the Idiopathic Pulmonary Fibrosis Clinical Research Network (IPFnet) research network at 26 sites throughout the USA PANTHER-IPF, STEP-IPF and ACE-IPF.

HRCT analysis
HRCT scans were obtained at 26 centres using between 2007 and 2012. Central radiology reading was not performed as part of the study entry process. The mean interval between the CT scan and SLBx was 1.75 years (range 0.01–10.4 years). When the maximal interval from CT scan to SLBx was limited to 1 year, 106 cases remained with a mean interval of 0.3 years (range 0.01–0.97 years). Two radiologists independently retrospectively reviewed the HRCT blind to clinical and histological information. Classified as UIP, possible UIP and inconsistent with UIP. The overall extent of the abnormalities was obtained by averaging the evaluations of the two independent observers. When they disagreed a consensus decision was made in collaboration with D.L. A higher threshold was applied for mosaic attenuation/air trapping, as these findings were required to be present bilaterally or in three or more lobes.

Pathological analysis: For the original network studies, a biopsy was reviewed by a trained pathologist at the study site using standard criteria “very similar” to the ATS/ERS/JRS/ALAT criteria and classified
as definite, probable, possible and not UIP; these results were then submitted to the pathology core for central reading. If the central read was discordant with the local site interpretation, a third pathologist was consulted, and all three pathologists conferred to reach a final conclusion. A small number of cases with discordant radiologic and pathologic findings were reviewed by a central adjudication committee, where a final determination of eligibility was made.

For the purposes of this study, cases were re-reviewed specifically to assess features that might correlate with the radiological findings that led to an ‘inconsistent’ CT interpretation. A subset of 100 cases, including (all available) 69 radiology-pathology discordant cases and two cases that were not UIP, were selected for an independent re-evaluation by two pathologists on the basis of one or two representative digitally scanned slides (as the initial actual slides had all been returned to the original site).

Presume that pathologists were blind to clinical and radiological findings. Where did the 2 not UIP cases come from?

Additional features that were evaluated on a semi-quantitative scale (0-3) included

- peribronchiolar metaplasia
- chronic inflammation and lymphoid hyperplasia
- giant cells
- bridging fibrosis
- organizing pneumonia/acute lung injury
- airway-centered interstitial changes, including inflammation and fibrosis (not accounted for by the presence of peribronchiolar metaplasia).

The scores of the two pathologists for each of these features were averaged.

Results

Baseline characteristics and PFTs of the study subjects

| TABLE 1 Clinical and physiological differences among the computed tomography diagnostic categories |
|---------------------------------|--------|--------|-----------------|--------|
| Subjects | Total | UIP | Possible UIP | Inconsistent with UIP |
| Sex male/female | 184/57 | 81/21 | 51/13 | 75/23 | 0.227 |
| Age years | 67.7±8.0 | 67.4±7.5 | 63.8±7.8 | 65.0±8.5 | 0.01 |
| Duration of illness years | 30±2.1 | 30±2.1 | 17±1.7 | 20±2.3 | 0.236 |
| Smoking ever/never | 179/6 | 62/20 | 42/22 | 51/24 | 0.065 |
| Post-walk Borg dyspnea score | 2.9±1.8 | 2.9±1.8 | 2.8±1.7 | 3.0±1.8 | 0.85 |
| St George’s Respiratory Questionnaire total score | 47.0±17.3 | 46.1±16.7 | 46.0±17.3 | 49.1±18.2 | 0.449 |
| 6-min walking distance m | 326.9±136.9 | 320.3±157.7 | 356.4±117.4 | 310.9±118.2 | 0.124 |
| Dco % pred | 37.8±13.4 | 35.3±13.6 | 41.7±14.8 | 38.6±11.9 | 0.01 |
| FEV1/FVC % | 77.5±19 | 77.3±16 | 77.9±16 | 77.9±23 | 0.029 |
| FVC % pred | 63.8±16.3 | 64.8±16.0 | 65.6±17.5 | 61.0±15.3 | 0.192 |
| Total lung capacity L | 4.0±1.1 | 4.1±1.1 | 4.2±1.2 | 3.7±0.9± | 0.011 |
| History of antigen exposure | 13 (5.39) | 8 (5.88) | 4 (6.25) | 3 (4.00) | 0.491 |
| Gastro-oesophageal reflux symptoms | 161 (64.8) | 64 (42.8) | 44 (68.8) | 53 (70.7) | 0.504 |

Data are presented as n, mean±sd or n (%), unless otherwise stated. UIP: usual interstitial pneumonia; Dco: diffusing capacity of the lung for carbon monoxide; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity. *: higher score indicates worse function. #: p<0.05 versus UIP group. \(^{\dagger}\): p=0.05 versus possible UIP group.

Radiologic diagnosis: Of the 241 cases-

- 102 (42.3%) - definite UIP
- 64 (26.6%) - possible UIP
75 (31.1%) - inconsistent with UIP

Comparison between radiological and pathological diagnoses

The inconsistent CT findings in the discordant group were due to:
- 51 (71.8%) - diffuse mosaic/air trapping
- 16 (22.5%) - extensive ground-glass abnormalities
- 17 (23.9%) - predominance of signs in the upper or mid-zones of the lungs
- 28 (39.4%) - diffuse craniocaudal distribution
- 9 (12.7%) - peribronchovascular predominance
- 19 (26.8%) - diffuse axial distribution.

Several subjects had more than one inconsistent CT finding

The results were similar when the analysis was restricted to the 106 individuals who underwent SLBx within 1 year of their CT scan

Evaluation of CT findings within the pathological diagnostic categories: There were no significant differences in CT findings between the pathological diagnoses
Physiological differences between radiology–pathology-concordant and -discordant groups

The individuals in the radiology–pathology-concordant group tended to be older, more likely to be current or former smokers, had lower FEV1/FVC and higher total lung capacity than those in the radiology–pathology-discordant group (table 6). When the analysis was restricted to the 106 individuals who underwent a SLBx within 1 year of the CT scan, the findings were similar (online supplementary table S4).
Detailed pathological evaluation between concordant and discordant groups

To assess the features that might correlate with the radiological findings that led to an “inconsistent” CT interpretation, subset of 100 cases, including (all available) 69 radiology-pathology discordant cases and a randomly selected set of 31 radiology-pathology concordant cases.

No significant differences were found in terms of the presence and degree of peribronchiolar metaplasia, chronic inflammation and lymphoid hyperplasia, giant cells, granulomas, bridging fibrosis, organising pneumonia/acute lung injury or airway-centered interstitial changes (online supplementary table S5).

<table>
<thead>
<tr>
<th>Table S5: Scores of the re-review of pathological findings in concordant and discordant cases.</th>
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<tbody>
<tr>
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<tr>
<td>Peribronchiolar metaplasia</td>
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<tr>
<td>Chronic inflammation</td>
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<tr>
<td>Giant cells</td>
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<tr>
<td>Granulomas</td>
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<tr>
<td>Bridging fibrosis</td>
</tr>
<tr>
<td>Organizing pneumonia/acute lung injury</td>
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<tr>
<td>Airway-centered interstitial changes</td>
</tr>
</tbody>
</table>

Data are presented as the mean score ± standard deviation.

12 radiology–pathology-concordant cases and 37 radiology–pathology-discordant cases had an interval of <1 year between their CT and lung biopsy, and, again, no significant differences were found between the concordant and discordant cases (online supplementary table S6).

Interobserver agreement among the two pathologists: peribronchiolar metaplasia κ=0.34; chronic inflammation and lymphoid hyperplasia κ=0.28; giant cells κ=0.29; granuloma κ=−0.04; bridging fibrosis κ=0.02; organising pneumonia/acute lung injury κ=0.41; and airway-centered interstitial changes κ=0.16.

Survival: The median duration of follow-up for subjects included in this study was ~ 1 yr.
38 patients died during the follow-up period. The results of the univariate Cox regression analysis regarding the relationship between survival and physiological or CT scan features are shown in online supplementary table S7.
Table S7: Predictors of survival on univariate analysis with Cox proportional hazard regression models.

<table>
<thead>
<tr>
<th>Predictor of survival</th>
<th>Hazard ratio</th>
<th>Predictor of outcome</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (male/female)</td>
<td>1.06</td>
<td>0.99-1.06</td>
<td>0.094</td>
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<tr>
<td>Sex (years)</td>
<td>1.55</td>
<td>0.408-10.09</td>
<td>0.553</td>
</tr>
<tr>
<td>Ever/never smoker</td>
<td>1.09</td>
<td>0.325-4.910</td>
<td>0.897</td>
</tr>
<tr>
<td>Reticular extent</td>
<td>1.26</td>
<td>0.65-2.28</td>
<td>0.487</td>
</tr>
<tr>
<td>Honeycombing</td>
<td>2.6</td>
<td>0.775-11.72</td>
<td>0.126</td>
</tr>
<tr>
<td>Honeycombing extent</td>
<td>1.35</td>
<td>0.85-1.99</td>
<td>0.191</td>
</tr>
<tr>
<td>Extensive ground glass abnormality</td>
<td>0.98</td>
<td>0.054-5.040</td>
<td>0.9839</td>
</tr>
<tr>
<td>Profuse micronodules</td>
<td>&lt;0.0001</td>
<td>3.125-3.125</td>
<td>0.2546</td>
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<tr>
<td>Diffuse mosaic attenuation/air-trapping</td>
<td>1.194</td>
<td>0.2649-4.006</td>
<td>0.7933</td>
</tr>
<tr>
<td>Emphysema</td>
<td>0.78</td>
<td>0.119-2.949</td>
<td>0.7375</td>
</tr>
<tr>
<td>Post walk Borg dyspnea score*</td>
<td>1.36</td>
<td>1.01-1.77</td>
<td>0.046</td>
</tr>
<tr>
<td>Total score on St. George’s Respiratory</td>
<td>1.08</td>
<td>1.04-1.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Questionnaire*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-minute walk distance (m)</td>
<td>0.99</td>
<td>0.98-0.99</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DLCO, % predicted</td>
<td>0.89</td>
<td>0.83-0.94</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEV1/FVC%</td>
<td>0.84</td>
<td>0.61-1.16</td>
<td>0.296</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>0.94</td>
<td>0.89-0.98</td>
<td>0.004</td>
</tr>
<tr>
<td>Total lung capacity</td>
<td>0.57</td>
<td>0.30-1.02</td>
<td>0.061</td>
</tr>
<tr>
<td>Concordant vs. discordant</td>
<td>1.90</td>
<td>0.549-8.699</td>
<td>0.322</td>
</tr>
</tbody>
</table>
In the univariate analysis, 6MWT, total score on SGRQ, post-walk Borg dyspnoea score, FVC % pred and DLCO % pred were significant predictors of survival (HR 0.99, 1.08, 1.36, 0.94 and 0.89, respectively). In the multivariate analysis, the total score on the SGRQ was the only significant predictor of mortality (HR 1.059).

The survival of the radiology–pathology-concordant group did not differ significantly from that of the radiology–pathology-discordant group (log-rank test p=0.334) during the follow-up period.

**Summary**

- **High discordance: 94.7% (71/75), inconsistent with UIP on HRCT** - definite or probable UIP on histology – likely falsely elevated as those patients who did not have UIP on histology initially were excluded from the original study. Authors state that “inconsistent with UIP” is a misleading term and patients with this radiological pattern should be biopsied.
- **29.3% (55/188) with a definite UIP pattern on histology had inconsistent with UIP on HRCT.** Similar to previous reports
- **Low concordance: 43.0% (99 / 230), UIP diagnosed on HRCT - definite or probable UIP on histology.** Low sensitivity similar to previous reports.

**Selection bias:** patients with a UIP pattern on HRCT do not usually undergo SLBx – perhaps those that did undergo biopsy were not as convincing on CT scan as those that were included in the study without biopsy?

With more cases of typical HRCT pattern included, the rates of concordant diagnosis between HRCT and pathology would increase.

Not generalizable to the broad population with fibrosing ILD as preselected and diagnosed at academic centres.

- **94% (60/64) possible UIP on HRCT had histologically definite or probable UIP.** The findings of possible UIP on HRCT may be diagnostic of UIP, and SLBx may not be necessary in these cases to confirm or clarify the diagnosis of IPF. But this study population was highly selected.

- Relatively high proportion with diffuse mosaic attenuation/air trapping in definite pathological UIP pattern, 21.3% (40 / 188). Careful histological re-review showed no evidence for hypersensitivity pneumonitis and concluded that most subjects actually had IPF – but no environmental evaluation done. **Current guidelines suggesting that these CT features are inconsistent with the diagnosis of IPF are probably too restrictive?**

- There was a relatively large observer variation for several of the HRCT and histological features-some of this variation could be due to the tendency of the κ-value to be reduced when the proportion of normal or abnormal findings is low.
Editorial:

“Ultimately, prospective multicentre longitudinal studies of all incident cases presenting with suspected IPF are required to better investigate clinical, radiological and molecular IPF phenotypes. This will provide the opportunity to increase our understanding of the natural history of chronic progressive fibrotic ILDs”

However, these results must be interpreted with caution if one is to avoid two inaccurate conclusions. First, that 95% of patients with a HRCT read as inconsistent with UIP have UIP and secondly, that 94% of patients with possible UIP on HRCT actually have UIP. The astute reader will realise that this study population is not generalisable to patients being investigated for suspected IPF in clinical practice. This is highly selected population of patients with an established diagnosis of IPF, taken from several clinical trial centres and without knowledge of the frequency of patients with inconsistent with UIP on HRCT who subsequently had a histological pattern other than UIP on biopsy; as a result, translating these findings directly to clinical practice is not possible. The term “inconsistent with UIP” be removed from future guidelines [6]. One possible replacement would be the term “radiologically inconsistent with UIP”, thus emphasising that in the absence of histopathological sampling UIP should not be confidently excluded.


Rationale: Surgical lung biopsy is often required for a confident multidisciplinary diagnosis of idiopathic pulmonary fibrosis (IPF). Alternative, less-invasive biopsy methods, such as bronchoscopic lung cryobiopsy (BLC), are highly desirable.

In their previous study, using BLC, the authors came to a diagnosis in 2/3 of the time. Does BLC provide useful information in the context of MDD?

Objectives: To address the impact of BLC on diagnostic confidence in the multidisciplinary diagnosis of IPF.

Methods: Cross-sectional study -117 patients with fibrotic ILD without a typical UIP pattern on HRCT identified from the ILDs database of Pneumology Unit at GB Morgagni Hospital, Forli, Italy, a referral center for ILDs. 58 BLC, and 59 SLB
Why were 12 patients in SLB group excluded from the study based on HRCT whereas non were excluded in BLC group? (eTable1)

The indication for SLB or BLC was a formal MDD made by the physicians that evaluated the patients at this center. Only four patients underwent both BLC and subsequent SLB.

The procedure was performed under deep sedation with a rigid tracheoscope and the cryobiopsies were obtained using a flexible cryoprobe (2.4 mm; ERBE, Tubingen, Germany) under fluoroscopic guidance. Two clinicians, two radiologists, and two pathologists sequentially reviewed clinical-radiologic findings and biopsy results, recording at each step in the process their diagnostic impressions and confidence levels.
Pathologic Assessment
Before the MDD 3 pathologists examined the pathologic specimens independently and in a blinded fashion recording their individual impressions and then discussed the cases and reached one final common pathology interpretation for each case and all agreed on the most likely diagnosis and on their global confidence level.

At least one fragment of alveolar lung parenchyma was required to classify the biopsy as adequate.

BLC was considered nondiagnostic when histopathologic criteria sufficient to define a characteristic histopathologic pattern were lacking. During the multidisciplinary meeting two pathologists were sequentially unblinded to clinical and radiologic data and again recorded their diagnostic impressions during the multistep MDD process.

The cases were reviewed by six experts in random order, with participants blinded as to the biopsy technique. The organizational scheme is summarized in Figure 1. The study was designed to evaluate whether the addition of BLC or SLB information influenced the diagnostic impression of participants (steps 5 and 6). The impact of bronchoalveolar lavage (BAL) (steps 3 and 4) and follow-up data (steps 7 and 8) were also evaluated in cases with available data.

Diagnosis was coded into six categories: (1) IPF, (2) idiopathic nonspecific interstitial pneumonia (NSIP), (3) desquamative interstitial pneumonia/respiratory bronchiolitis ILD, (4) hypersensitivity pneumonitis, (5) other, and (6) unclassifiable. Confidence levels (low, intermediate, or high) were recorded.
Measurements and Main Results:

As shown in Figure 2 the major change in diagnostic confidence occurred with the addition of histologic information, and this applied equally for BLC and SLB. Adding BLC/SLB information to clinical-radiologic data increased the confidence level in most suspected IPF cases (78% in both) and the prevalence of IPF diagnosis made with high level of confidence doubled from 29 to 63% with BLC (P = 0.0003) and from 30 to 65% with SLB (P = 0.0016) (Figure 2).
The overall interobserver agreement in IPF diagnosis was similar for both (BLC, 0.96; SLB, 0.93). IPF was the most frequent diagnosis (50 and 39% in the BLC and SLB group, respectively; P = 0.23). After the addition of histopathologic information, 17% of cases in the BLC group and 19% of cases in the SLB group, mostly NSIP and HP, were reclassified as IPF. 4 cases (BLC, subsequent SLB), 1 NSIP, 1 DIP, 1 IPF, 1 BLC unclassifiable, SLB diagnosis was chronic HP.
Change in diagnosis after histology added
15/58 (26%) for BLC
22/59 (37%) for SLB
Pathologists’ independent interpretations of SLB and BLC specimens. Biopsy specimens were considered “adequate” when containing at least one alveolated fragment and “diagnostic” when containing features sufficient to define a pathologic pattern. Pathologists’ confidence levels in their blind identification of UIP were stratified into two groups, high (H) and low (L). Kappa value measures the interobserver agreement among the three pathologists for the discrimination between UIP and non-UIP cases.

Nondiagnostic: BLC – 91%, SLB- 98%
When UIP was diagnosed at biopsy with high confidence, the final diagnosis was IPF in 90% of BLC and in 63% of SLB.

**BUT IPF final:** 50% of BLC and only 39% of SLB

**Safety of BLC**
Pneumothorax - 33% (19 of 58), with most (15 of 19) requiring chest tube drainage. Patients with pneumothorax required hospitalization.
BLC 1 death
SLB 2 deaths
all IPF acute exacerbation.

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<table>
<thead>
<tr>
<th>Clinical review (step 2)</th>
<th>Clinical review plus BLC (step 6)</th>
<th>p value</th>
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<tbody>
<tr>
<td>3 IPF-H</td>
<td>3 IPF-H</td>
<td>1</td>
</tr>
<tr>
<td>11 IPF-I</td>
<td>10 IPF-H</td>
<td>0.596</td>
</tr>
<tr>
<td>4 IPF-L</td>
<td>3 IPF-H</td>
<td>0.444</td>
</tr>
<tr>
<td>31 non-IPF</td>
<td>2 IPF-H</td>
<td>0.848</td>
</tr>
<tr>
<td>9 no consensus</td>
<td>1 IPF-H</td>
<td>0.914</td>
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<td>0 unclassifiable</td>
<td>NA</td>
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<th>Clinical review plus SLB (step 6)</th>
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</table>
Issues with design/report of study:
- Exclusion of subjects in SLB prior to study
- Initially included 3 pathologists and then just 2
- Could not find details on numbers and size of cryobiopsies or SLBs
- Very selective regarding which measurements were reported

Conclusions:
- BLC increases diagnostic confidence in the MDD diagnosis of IPF with a similar impact to SLB on the MDD process. **DOES IT?**
- No conclusion can be drawn about the diagnostic accuracy of BLC compared with SLB.
- Pathologists were less confident in their independent revision of BLC compared with SLB
  - **AGREE**
  - UIP–high confidence diagnosis (85% SLB, 52% BLC)
  - higher interobserver agreement in the SLB (0.86 compared with 0.59 of BLC).
  - pathologists with previous experience in BLC analysis had an agreement nearly equivalent to SLB (0.73 for BLC, compared with 0.86 for SLB).
- “ Seems safe”. **IS IT?**

Limitations:
- referral center for ILDs, and doesn’t capture the complexity of real-life MDD
- BLC led to a change in the initial clinical–radiologic diagnosis in 26% of cases (compared with about 37% of those undergoing SLB)
- Exclusion of 12 patients from SLB group based on HRCT diagnosis of UIP and not from BLC group may have biased study in that patients in SLB group had classic less features of UIP and have led to higher diagnostic rate of IPF in BLC than in SLB
- small central samples from a single segment may not provide the same qualitative data as multiple peripheral samples obtained from multiple lobes.
- Final IPF in 50% BLC only 39% SLB? cryobiopsy may miss important qualitative information in unsampled lobes that would go against diagnosis of IPF
- The lower agreement between pathologists in identifying UIP in cryobiopsy

Safety:
- 1/3 developed pneumothoraces, higher than seen in previous studies – no severe bleeding but this has been seen other studies
- Cannot assume that cryobiopsy will have less risk in those patients who are at higher risk of complications for biopsy

Editorial:
Patel et al. **Cryobiopsy in the Diagnosis of Interstitial Lung Disease A Step Forward or Back?**

“The complication rate in one single-center study was 19% (16), and the mortality rate was 0.6% in nonimmunocompromised adults with ILD in a large meta-analysis (17). In our experience, much of the risk for surgical biopsy can be avoided by carefully selecting candidates without advanced disease, extensive parenchymal or pleural fibrosis, or significant pulmonary hypertension”.
“The implementation of cryobiopsy for those at high risk for complications from a surgical lung biopsy also requires study: their risk may be high for complications from cryobiopsy as well. The investigation of alternative diagnostic strategies that provide less data (smaller biopsy samples) when we already have inadequate diagnostic tools, a lack of clear evidence-based diagnostic approaches and guidelines, and a subjective, labor-intensive, “less than gold” gold standard could be a step backward”.


**Objective** - Transbronchial cryobiopsy technique yields larger biopsies with enhanced quality. The benefits and safety of cryobiopsies have not been thoroughly studied in lung allografts.

**Design**
- All cryobiopsies (March 2014–January 2015) of lung allografts performed at Mayo Clinic, Rochester, and medical records were reviewed.
- cryobiopsy or conventional biopsy at the operator’s discretion
  - largely based on experience and comfort level of bronchoscopist
  - risk for bleeding - conventional biopsy
- For comparison, conventional biopsies from the same patient or, if unavailable, from a random patient, were selected.
- Two pathologists blinded to outcome reviewed all biopsies.
- Discrepant cases were reviewed by a third lung transplant pathologist.
- Cell-mediated and small airways rejection was graded according to the 2007 working formulation of lung rejection of the ISHLT.
- Specimen volume, number of alveoli, small airways, and pulmonary vessels were counted and statistically compared.
- Biopsies were evaluated for crush artifact and atelectasis and evaluated for acute hemorrhage within alveoli, which was regarded as a procedural artifact.

**Results** - Fifty-four biopsies (27 cryobiopsies) from 18 patients (11 men) were reviewed. A median of 3 (range, 2–5) and 10 (range, 6–12) specimens were obtained with cryobiopsies and conventional biopsies, respectively.
Table 2. Summary of Clinical Characteristics of Patients and Histopathologic Findings Based Upon Type of Allograft Transbronchial Biopsy Performed

<table>
<thead>
<tr>
<th></th>
<th>Cryobiopsies (n = 27)</th>
<th>Conventional Biopsies (n = 27)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time between transplantation and biopsy, median (range), d</td>
<td>314 (29-5516)</td>
<td>184 (29-4364)</td>
<td>.02</td>
</tr>
<tr>
<td>No. (%)</td>
<td></td>
<td></td>
<td>.58</td>
</tr>
<tr>
<td>Protocol biopsies</td>
<td>10 (37.0)</td>
<td>8 (29.6)</td>
<td></td>
</tr>
<tr>
<td>Indication biopsies</td>
<td>17 (63.0)</td>
<td>19 (70.4)</td>
<td></td>
</tr>
<tr>
<td>Volume of tissue per biopsy, median (range), cm³</td>
<td>0.50 (0.06-3.07)</td>
<td>0.13 (0.02-0.64)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No. of alveoli, median (range)</td>
<td>5228 (1967-11 336)</td>
<td>2469 (639-6973)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No. of small airways per biopsy, median (range)</td>
<td>2 (0-6)</td>
<td>1 (0-4)</td>
<td>.04</td>
</tr>
<tr>
<td>Biopsies without small airways, No. (%)⁶</td>
<td>3 (11.1)</td>
<td>7 (25.9)</td>
<td>.28</td>
</tr>
<tr>
<td>Biopsy specimens from patients with BOS at time of biopsy, No. (%)</td>
<td>9 (33.3)</td>
<td>7 (25.9)</td>
<td>.31</td>
</tr>
<tr>
<td>No. of biopsies with ISHLT grade C1 from patients with BOS at time of biopsy</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No. of pulmonary arteries, median (range)⁷</td>
<td>2 (0-7)</td>
<td>2 (0-7)</td>
<td>.83</td>
</tr>
<tr>
<td>No. of pulmonary veins, median, (range)⁷</td>
<td>9 (3-16)</td>
<td>5.5 (0-12)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Biopsies with acute (likely procedure-related) hemorrhage, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19 (70.4)</td>
<td>1 (3.7)</td>
<td>Reference</td>
</tr>
<tr>
<td>Focal</td>
<td>6 (22.2)</td>
<td>14 (51.9)</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>Diffuse</td>
<td>2 (7.4)</td>
<td>12 (44.4)</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>Biopsies with crush artifact/atelectasis, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19 (70.4)</td>
<td>1 (3.7)</td>
<td>Reference</td>
</tr>
<tr>
<td>Focal</td>
<td>8 (29.6)</td>
<td>10 (37.0)</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>Diffuse</td>
<td>0</td>
<td>16 (59.3)</td>
<td></td>
</tr>
<tr>
<td>Biopsies without or with diffuse crush artifact/atelectasis and/or hemorrhage, No. (%)</td>
<td>14</td>
<td>23</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>No artifact or hemorrhage</td>
<td>12 (45.7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Diffuse artifact and/or hemorrhage</td>
<td>2 (7.4)</td>
<td>23 (100.0)</td>
<td></td>
</tr>
<tr>
<td>Biopsies with additional findings, No. (%)</td>
<td>14 (51.9)</td>
<td>11 (40.7)</td>
<td>.61</td>
</tr>
<tr>
<td>Large airway inflammation</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Focal organizing pneumonia</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Granuloma</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Thrombi</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Calcifications</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Focal pulmonary alveolar proteinosis</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Focal fibrosis</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Acute lung injury</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Intimal proliferation of artery versus oblitative bronchiolitis ⁸</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Focal lymphocytic infiltrate, not sufficient for ACR</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Procedural complications, No. (%)</td>
<td>7 (25.9)</td>
<td>4 (14.8)</td>
<td>.25</td>
</tr>
<tr>
<td>Mild bleeding</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Moderate bleeding</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Severe bleeding</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Delayed hemorrhax</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Delayed pneumothorax</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Intubation post bronchoscopy</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ACR, acute cellular rejection; BOS, bronchiolitis obliterans syndrome; ISHLT, International Society for Heart and Lung Transplantation.

⁴ Information available on 25 cryobiopsies and all conventional biopsies.
⁵ As defined by reviewer A.
⁶ Data available from 26 cryobiopsies and 24 conventional forceps biopsies. Verheo-FF Van Gieson stain failed in the other biopsies.
⁷ Focal versus none.
⁸ Diffuse versus none.
⁹ Cannot be calculated with generalized estimating equation methodology because of sparse data (zero cell count).
¹⁰ Includes only biopsies with "none" on both or "diffuse" on one or the other. Excludes biopsies with "focal."
¹¹ In a biopsy with extensive crush artifact.
Cryobiopsies were larger and contained more alveoli \((P < .001, \text{both})\) and small airways \((P = .04)\). Conventional biopsies showed more fresh alveolar hemorrhage (procedural) and crush artifact/atelectasis \((P < .001, \text{both})\). Cryobiopsies contained more pulmonary veins and venules \((P < .001)\).

There was no significant difference between the types of biopsies with respect to the reviewers’ agreement on grades of rejection.

While 9 cryobiopsies and 7 conventional biopsies were from patients who had BOS at the time of biopsy, none of these biopsies were morphologically recognized as ISHLT grade C1 rejection by any reviewer although C1 rejection was noted in cases that did not have any evidence of BOS at time of biopsy (in 3 cryobiopsies and 3 conventional biopsies).

**Figure 2.** Boxplots of volume (A) and number of alveoli (B) of cryobiopsies and conventional biopsies. The horizontal line in the middle of a shaded box represents the median; the lower and upper ends of a shaded box are the 25th and 75th percentiles (Q1 and Q3); and individual points represent observations that fall beyond a distance equal to 1.5 \times \text{(interquartile range)} beyond Q1 or Q3.

<table>
<thead>
<tr>
<th>ISHLT Grade of Rejection</th>
<th>Cryobiopsy, No. (%) (n = 27)</th>
<th>Conventional Biopsy, No. (%) (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0</td>
<td>12 (44.4)</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td>A1</td>
<td>17 (63.0)</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td>A2</td>
<td>20 (71.4)</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td>A3</td>
<td>2 (7.4)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>A4</td>
<td>5 (18.5)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>B0/BX</td>
<td>25 (92.6)</td>
<td>22 (81.5)</td>
</tr>
<tr>
<td>B1</td>
<td>2 (7.4)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>B2</td>
<td>5 (18.5)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>C0/CX</td>
<td>24 (88.9)</td>
<td>22 (81.5)</td>
</tr>
<tr>
<td>C1</td>
<td>25 (92.6)</td>
<td>22 (81.5)</td>
</tr>
<tr>
<td>D0</td>
<td>21 (77.8)</td>
<td>25 (92.6)</td>
</tr>
<tr>
<td>D1</td>
<td>6 (22.2)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviation: N/A, not applicable.
Quality of biopsies:
- Pathologists agreed that cryobiopsies were much easier to interpret
- no crush artifact/atelectasis
  - 19/27 (70.4%) cryobiopsies
  - 1/27 (3.7%) conventional biopsies
- no acute hemorrhage
  - 19/27 (70.4%) cryobiopsies
  - 16/27 (3.7%) conventional biopsies
- Diffuse crush artifact/atelectasis
  - 0/27 (0%) cryobiopsies
  - 16/27 (59.3%) conventional biopsies
- Small airways
  - Significantly more in cryobiopsies but not present in all

Complications (table 2)
- more frequent in the cryobiopsy group, (not statistically significant).
- 1- intubation and mechanical ventilation post conventional biopsy for 6 days - respiratory failure.
- 1- delayed pneumothorax after cryobiopsy but an endobronchial mass had been removed
- 1- delayed large unilateral hemothorax after cryobiopsy. That patient had scleroderma and issues with DVT

Conclusions
- Cryobiopsies of lung allografts are larger and have less artifact
- Relatively low variability of data (most no rejection) may account for inability to demonstrate superiority of cryobiopsy data.
- complications occur and should be considered.
- Although the volume of tissue with cryobiopsy 4-fold higher, number of alveoli, small airways, and veins and venules 2-fold higher – it is the 2 dimensional features that count for diagnostic purposes
- 3 cryobiopsy specimens may be adequate as ISHLT guidelines “at least five pieces of well-expanded alveolated lung parenchyma are required to assess acute rejection.”
- Suggestions for which patients should undergo cryobiopsy rather than conventional
  - for patients for whom clinical suspicion of rejection persists after negative conventional forceps biopsy results
  - when the morphologic diagnosis is equivocal
  - when concern for obliterative bronchiolitis.

Limitations: (all pointed out by authors)
- Bias in whether cryobiopsy or conventional performed
  - Skill/other of operator
  - Bleeding risk
  - ?perception of other issues
- Cannot blind pathologist to which biopsy has been performed
Larger studies needed to assess superiority or otherwise of cryobiopsies due to low variability of data – cryobiopsy and conventional at the same time preferred (clinical trial is being undertaken, NCT01694615)

- Larger studies needed to assess safety
- Long-term studies also needed to assess if there are late complications e.g. fibrosis

**Articles for Notation**

**Neoplastic**


**Context**. - The separation of benign from malignant mesothelial proliferations is crucial to patient care but is frequently morphologically difficult.

**Objective**.- To briefly review adjunctive tests claimed to be useful in this setting and to examine in detail 2 new tests: p16 fluorescence in situ hybridization (FISH) and BRCA1-associated protein 1 (BAP1) immunohistochemistry.

**Design**.- Literature review with emphasis on p16 FISH and BAP1 immunohistochemistry.

**Results**.- Glucose transporter-1, p53, insulin-like growth factor 2 messenger RNA–binding protein 3 (IMP-3), desmin, and epithelial membrane antigen have all been claimed to mark either benign or malignant mesothelial processes, but in practice they at best provide statistical differences in large series of cases, without being useful in an individual case. Homozygous deletion of p16 by FISH or loss of BAP1 has only been reported in malignant mesotheliomas and not in benign mesothelial proliferations. BAP1 appears to be lost more frequently in epithelial than mixed or sarcomatous mesotheliomas. Homozygous deletion of p16 by FISH is seen in pleural epithelial, mixed, and sarcomatous mesotheliomas, but it is much less frequent in peritoneal mesothelioma. The major drawback to both these tests is limited sensitivity; moreover, failure to find p16 deletion or BAP1 loss does not make a mesothelial process benign.

**Conclusions**.- In the context of a mesothelial proliferation, the finding of homozygous deletion of p16 by FISH or loss of BAP1 by immunohistochemistry is, thus far, 100% specific for malignant mesothelioma. The limited sensitivity of each test may be improved to some extent by running both tests.

**Take home message:**

- Nice review which persuasively presents data against using glucose transporter-1, p53, IMP-3, desmin, and EMA in individual cases of mesothelial proliferations.
- So far, p16 deletion by FISH or BAP1 loss by IHC have not been detected in benign mesothelial lesions.
- These markers are of value if they are positive but they have limited sensitivity and both should be used if one is negative.
- It should be remembered that their loss is not limited to mesothelioma but can occur in other malignancies.
- In most cases we are still stuck with the appearance on morphology and whether or not there is invasion into fat etc.

**Introduction** - Recent regulatory changes have allowed the diagnostic use of immunohistochemical (IHC) analysis for the identification of patients with non–small cell lung cancer who are eligible for treatment with anaplastic lymphoma receptor tyrosine kinase (ALK) inhibitors. The U.S. Food and Drug Administration has approved the VENTANA ALK (D5F3) CDx Assay (Ventana Medical Systems, Tucson, AZ) as companion diagnostics, and the Italian Medicines Agency has recognized IHC analysis as a diagnostic test indicating an algorithm for patient selection.

**Methods** - On the basis of the new regulations, we compared two commonly used IHC assays on 1031 lung adenocarcinomas: the VENTANA ALK (D5F3) CDx Assay with the OptiView Amplification Kit (Ventana Medical Systems) and a standard IHC test with the clone 5A4 (Novocastra, Leica Biosystems, Newcastle Upon Tyne, United Kingdom) along with their interpretative algorithms. Fluorescence in situ hybridization (FISH) was performed in all cases. Next-generation sequencing was performed in FISH/IHC analysis–discordant samples.

**Results** - FISH gave positive results in 33 (3.2%) cases. When FISH was used as a reference, the VENTANA ALK (D5F3) CDx assay had a sensitivity of 90.9% ± 2.6%, a specificity of 99.8% ± 0.6%, and positive and negative predictive values of 93.8% ± 2.1% and 99.7% ± 0.6%, respectively. The clone 5A4–based IHC test showed a sensitivity of 90.9% ± 2.6%, a specificity of 98.3% ± 1.3%, and positive and negative predictive values of 63.8% ± 4.2% and 99.7% ± 0.6%, respectively. Five cases with IHC analysis/FISH-discordant results in our series were analyzed together with those previously reported in the literature. Overall, data from 35 patients indicate a response rate to ALK inhibitors in 100% of FISH-negative/IHC analysis–positive cases (seven of seven) and 46% of FISH-positive/IHC analysis–negative cases (13 of 28), respectively.

**Take home message:**
- A negative test by IHC for ALK would trigger a FISH test depending on the clinical status (younger age, non-smoker), and histologic type (mucinous cribriform pattern and signet ring cell carcinoma and positive TTF1/p63)
- Acknowledges that FISH is not the gold standard anymore as a response rate to ALK inhibitors occurred in 100% of FISH-negative/IHC analysis–positive cases

Matsuzawa et al. Factors influencing the concordance of histological subtype diagnosis from biopsy and resected specimens of lung adenocarcinoma. Lung Cancer 2016; 94: 1-6

**Objectives** - Lung adenocarcinoma is heterogeneous, characterized by various histological subtypes. Determination of the predominant histological subtype (lepidic, papillary, acinar or solid-predominant) has been shown to correlate with genetic abnormalities and clinicopathological features. Although subtyping using small biopsy samples is important for tailored approaches to clinical management, limited data exist on the concordance of predominant subtype between resected specimens and biopsy specimens.

**Materials and methods** - We compared the diagnosed predominant subtypes in resected specimens and matched biopsy specimens in a series of 327 lung adenocarcinomas. The accuracy of preoperative diagnosis by biopsy and the factors that influence concordance with resected specimen analysis were examined.
Results - In 211 of the 326 patients (66.0%), the predominant adenocarcinoma subtype diagnosed from biopsy matched the findings of resection analysis. Overall, the concordance rate in biopsy samples with larger tumor areas ($\geq 0.7 \text{ mm}^2$) was significantly higher than in those with smaller tumor area ($<0.7 \text{ mm}^2$; 71.2% vs 60.7%, respectively; $p = 0.015$). In the biopsy samples with smaller tumor areas, the concordance rate was 77% in lepidic subtype, 71% in papillary subtype, 60% in solid subtype, and 40% in acinar subtype. Concordance rate in the biopsy samples with larger tumor area was higher in papillary and solid subtypes (88% and 76%, respectively), but remained low in acinar subtype (37%).

Take home message:
A rather predictable outcome. Limited by the pathologist specific factors. With the tendency to obtain smaller samples, it is useful to remember that treatment based on such small samples might not be optimal.


Context.—Although epidermal growth factor receptor (EGFR)– and anaplastic lymphoma kinase (ALK)–directed therapies are not approved for patients with early-stage non–small cell lung carcinoma (NSCLC), many institutions perform EGFR and ALK testing for all patients with NSCLC at the time of initial diagnosis. Current consensus guidelines recommend EGFR testing and suggest ALK testing at the time of initial diagnosis for patients with advanced disease.

Objectives.—To examine the cost and clinical impact of EGFR and ALK testing of patients with early-stage NSCLC.

Design.—Records from all patients with a diagnosis of NSCLC made on a nonresection specimen at our institution during a single calendar year (2012) were reviewed, and a cost analysis was performed.

Results.—Of 133 total patients, 47 (35%) had early stage (stage I or II) disease and 86 (65%) had locally advanced (stage III) or advanced (stage IV) disease at presentation. Eight of 47 patients with early-stage disease (17%) had progression/recurrence during 18 to 30 months of follow-up, 6 of 8 (75%) of whom had pathologic confirmation of progression/recurrence. The estimated additional cost of EGFR and ALK testing for all newly diagnosed patients with NSCLC at our institution is $75 200 per year, compared to testing only patients with locally advanced and advanced-stage disease.

Take home message: A good argument against universal testing as most patients do not have disease recurrence/progression. This has to be balanced against the possibility of a sample not being available (lost, misfiled etc) if a patient progresses but should be an unusual situation and a re-biopsy could be performed.

Pelosi1 et al. Doing more with less: fluorescence in situ hybridization and gene sequencing assays can be reliably performed on archival stained tumor tissue sections. Virchows Arch 2016; 468: 451-461

Background: Little is known about molecular testing on tumor tissue retrieved from stained sections, for which there may be a clinical need.
**Methods:** We retrospectively analyzed 112 sections from 56 tumor patients using either fluorescence in situ hybridization (FISH) with different probes (19 sections from 17 patients) or Sanger or targeted next generation sequencing for detection of BRAF, EGFR, KRAS, C-KIT, and TP53 mutations (93 sections from 39 patients). Tumor tissue sections had been stained by hematoxylin and eosin (H&E) (42 sections) or by immunohistochemistry for cytoplasmic or nuclear/nuclearcytoplasmic markers (70 sections) with a peroxidase (P-IHC, with 3,3′-diaminobenzidine as chromogen) or alkaline phosphatase label (AP-IHC, with Warped™ as chromogen).

**Results:** For FISH analysis, the concordance rate between the original diagnosis and that obtained on H&E- or P-IHC-stained tissue sections (AP-IHC was not on record for this set of patients) was 95 % (18 out of 19 tumor sections). Only one tumor sample, diffusely positive for MLH1, did not yield any nuclear hybridization signal. For sequencing analysis, the concordance rate was 100 % on negative P-IHC and positive AP-IHC-stained sections, regardless of the subcellular localization of the reaction product. Mutations were detected in only 52 % of cases expressing nuclear/nuclear-cytoplasmic markers, regardless of the sequencing technology used (p = 0.0002).

**Take home message:** With the increase in targets for therapy, samples for molecular testing are more likely to be inadequate. Useful study which confirms that archival stained tumor tissue can be re-used. Encouraging results that show that hematoxylin does not interfere, with the caveat that DAB may be a problem with retesting especially if it stained the nucleus.


**Introduction:** Activating mutations in the epidermal growth factor receptor gene (EGFR) predict for prolonged progression-free survival in patients with advanced non–small cell lung cancer (NSCLC) treated with EGFR tyrosine kinase inhibitors (EGFR-TKIs) versus chemotherapy. Long-term survival outcomes, however, remain undefined. The objective of this study was to determine the 5-year survival in these patients and identify clinical factors associated with overall survival (OS).

**Methods:** Patients with EGFR-mutant metastatic lung adenocarcinoma who had been treated with erlotinib or gefitinib at Dana-Farber Cancer Institute between 2002 and 2009 were included. OS was analyzed.

**Results:** Among 137 patients, median progression-free survival and OS were 12.1 months (95% CI: 10.2–13.5) and 30.9 months (95% CI: 28.2–35.7), respectively. Twenty patients (14.6%) were 5-year survivors. In multivariate analysis, exon 19 deletions (hazard ratio [HR]¼ 0.63, 95% CI: 0.44–0.91, p¼0.01), absence of extrathoracic (HR ¼ 0.41–0.93, p¼0.02) or brain metastasis (HR ¼ 0.48, 95% CI: 0.30–0.77, p¼0.002), and not a current smoker (HR¼0.23,95%CI: 0.09–0.59, p ¼ 0.002) were associated with prolonged OS. Age; sex; stage at diagnosis; liver, bone, or adrenal metastasis; specific TKI; and line of TKI therapy were not associated with OS.

**Conclusions:** The rate of 5-year survival among patients with EGFR-mutant metastatic lung adenocarcinoma treated with erlotinib or gefitinib in this study was 14.6%. Exon 19 deletions and absence of extrathoracic or brain metastasis are associated with prolonged survival.

**Take home message:** I included this study as I was interested to know if targeted therapies are having an impact on longer term survival. Study is limited by being retrospective and relatively small.

**Context.**—TTF-1 and napsin A immunomarkers have a crucial role in differentiating lung adenocarcinoma from lung squamous cell carcinoma and in identifying a primary lung adenocarcinoma when working on a tumor of unknown origin.

**Objectives.**—To investigate the diagnostic sensitivity of ribonucleic acid in situ hybridization (RNAscope) in the detection of expression of these biomarkers in lung adenocarcinomas and to compare RNAscope to immunohistochemical techniques.

**Design.**—Both RNAscope and the immunohistochemical assays for TTF-1 and napsin A were performed on tissue microarray sections containing 80 lung adenocarcinomas and 80 lung squamous cell carcinomas. The RNAscope assay for both TTF-1 and napsin A was also performed on 220 adenocarcinomas from various organs.

**Results.**—The RNAscope assay for TTF-1 gave positive results in 92.5% (74 of 80) of the lung adenocarcinomas; in contrast, immunohistochemistry gave positive results in 82.5% (66 of 80) of those cases. The RNAscope assay for napsin A gave positive results in 90% (72 of 80) of lung adenocarcinomas; immunohistochemistry results were positive in 77.5% (62 of 80) of those cases. Napsin A expression was not seen in lung squamous cell carcinomas by either method. In contrast, TTF-1 expression was seen in 3.8% (3 of 80) (1þ) and 10% (8 of 80) (1þ) of the squamous cell carcinomas by immunohistochemistry and the RNAscope, respectively. All nonpulmonary adenocarcinoma results were negative for TTF-1 by the RNAscope assay.

**Take home message:** In small letters on the first page of the article, it is mentioned that: “Drs Ma, He, and Luo are employees and stockholders of Advanced Cell Diagnostics, Inc. The RNA probes for TTF-1 and napsin A, related reagents, and the HybEZ oven are products of that company”. The paper doesn’t specify what these authors role in the paper was. I would have had more confidence in the outcomes if these authors had not been included in the study. Only the Ventana Ultra IHC platform was used, therefore the results are not generalizable to all IHC tests.


**Aims:** The role of tumour metabolic and proliferative indices in predicting non-small-cell lung cancer (NSCLC) patients’ prognosis is unclear. We correlated fluorine 18 (18F)-fluorodeoxyglucose (FDG)-positron emission tomography (PET) value and Ki67 index to patients’ survival, taking into account tumour heterogeneity, disease characteristics and genetic aberrations.

**Methods and results:** A series of 383 NSCLCs was arranged into tissue microarrays and Ki67 staining was analysed by immunohistochemistry. The maximum standardized uptake (SUVMAX) value detected by 18F-FDG-PET analysis was calculated over a region of interest. Large-cell and squamous cell carcinomas had higher proliferative and metabolic activities than adenocarcinomas, and the two measures were correlated significantly. The hot-spot Ki67 value was correlated with patients’ survival and the cut-off to discriminate patients in the survival risk groups was 20%. Ki67 hot-spot values were greater in anaplastic lymphoma kinase (ALK) rearranged tumours. Adenocarcinomas showed the highest intratumour heterogeneity in proliferative activity and the hot-spot Ki67 value predicted only the prognosis of patients in this group. Although tumour metabolic activity was not associated with patients’ prognosis, a SUVMAX > 2 was related to nodal metastases, tumour size and grade.
Take home message: The authors state that their data “supports Ki67 evaluation to estimate NSCLC patients’ prognosis, particularly for adenocarcinoma,” however, Ki67 was NOT an independent variable predicting prognosis.


Context.—The classification of pulmonary large cell carcinoma has undergone a major revision with the recent World Health Organization (WHO) 2015 Classification. Many large cell carcinomas are now reassigned to either adenocarcinoma with solid pattern or nonkeratinizing squamous cell carcinoma based on immunopositivity for adenocarcinoma markers or squamous cell carcinoma markers, respectively. Large cell carcinomas that are negative for adenocarcinoma and squamous cell carcinoma immunomarkers are now classified as large cell carcinoma with null immunohistochemical features (LCC-N). Although a few studies investigated the mutation profile of large cell carcinomas grouped by immunostain profile before the publication of the new WHO classification, investigation of tumors previously diagnosed as large cell carcinoma and reclassified according to the 2015 WHO classification has not, to our knowledge, been reported. Objective.—To determine the mutation profiles of pulmonary large cell carcinomas reclassified by WHO 2015 criteria.

Design.—Archival cases of non–small cell lung carcinoma with large cell carcinoma morphology (n = 17) were reclassified according to 2015 WHO criteria. To determine mutation profile, we employed Ion Torrent (Life Technologies, Carlsbad, California)–based next-generation sequencing (50 genes; more than 2800 mutations) in addition to real-time quantitative reverse transcription polymerase chain reaction for ALK translocation detection.

Results.—Two of 17 cases (12%) were reclassified as LCC-N, and both had mutations—BRAF D594N in one case and KRAS G12C in the other case. Seven of 17 cases (41%) were reclassified in the adenocarcinoma with solid pattern group, which showed one KRAS G12C and one EGFR E709K + G719C double mutation in addition to mutations in TP53. Eight of 17 cases (47%) were reclassified in the nonkeratinizing squamous cell carcinoma group, which showed mutations in PIK3CA, CDKN2A, and TP53. No ALK translocations or amplifications were detected.

Take home message: No surprises, it has been shown before that most large cell carcinomas can be classified as squamous or adenocarcinoma, but is nice to get further confirmation.


A nice short review on the impact of low-dose computed tomography screening for lung cancer, the new WHO classification of lung cancers, and IHC based companion diagnostics on pathology.

This review is recommended background reading to prepare for the various targeted therapies (and therefore companion tests) that are coming down the pipeline in the future.

**PDL-1 related reviews:**

**Neoplastic Case Reports**


Interesting analysis of this rare tumor


**Aims:** Seven cases of primary giant cell carcinomas of the lung are presented.

**Methods and results:** The patients were five women and two men between the ages of 48 and 72 years (average: 63 years). Clinically, the patients presented with symptoms of cough, chest pain, dyspnoea and general malaise. Diagnostic imaging revealed the presence of intrapulmonary masses; five tumours were located in the right lung and two in the left, with a general predilection for the upper lobes. All patients underwent surgical resection and staging of their tumours. Five patients were staged as T2 and T3 with nodal metastasis, while two patients in stages T1 and T3, respectively, had no nodal disease. Histologically, the giant cells were typed as syncytiotrophoblast-like or ‘null type’, according to the expression of b-human chorionic gonadotrophin or expression of cytokeratin alone. Follow-up information revealed that five patients died within a period of 1–3 years, while two patients remain alive between 1 and 3 years.

**Take home message:** Although a very small number of cases, the authors do present a convincing argument for classifying these tumors separately from sarcomatoid carcinomas and this warrants further study.
Non-neoplastic:


A working group created by the ISHLT has published consensus criteria for AMR for the first time.


A nice up to date review of pathophysiological, diagnostic, prognostic, and therapeutic features of pulmonary MALT lymphoma.


A nice review of pathophysiology, pathology and clinical features but very little new to help predict morbidity and mortality.


Objective - Lung transplantation is the ultimate treatment for end-stage pulmonary sarcoidosis. Post-transplant survival outcomes remain unclear.
Methods - Survival models were used to assess survival and graft outcomes in patients with sarcoid among 20 896 lung transplants performed in the USA.
Results - 695 lung recipients were transplanted for pulmonary sarcoidosis. Sarcoid lung recipients had similar median survival rate (69.7 months (IQR 60.2–79.3)) compared with the nonsarcoid lung recipients (63.1 months (IQR 61.4–64.8), p=0.88). In multivariate Cox regression, sarcoidosis was not independently associated with worse mortality (HR 0.96 (95% CI 0.85 to 1.08), p=0.51). Among the sarcoid lung recipients, double lung transplantation (HR 0.76 (0.58 to 0.99), p=0.04) and lung allocation score era (HR 0.74 (0.56 to 0.97), p=0.03) were associated with improved survival.

Take home message: Recipients of lung transplants for pulmonary sarcoidosis had similar outcomes compared with non-sarcoid lung recipients.

Letters relating to a previous article on IPAF: Let the debate continue.
Collins et al. Interstitial pneumonia with autoimmune features: the new consensus-based definition for this cohort of patients should be broadened. Eur Respir J 2016; 47: 1292-1293
Fischer et al. Interstitial pneumonia with autoimmune features: the new consensus-based definition for this cohort of patients should be broadened. Eur Respir J 2016; 47: 1293-1295
Non-neoplastic Case Reports:


Context: Giant cell interstitial pneumonia is a rare lung disease and is considered pathognomonic for hard metal lung disease, although some cases with no apparent hard metal (tungsten carbide cobalt) exposure have been reported. We aimed to explore the association between giant cell interstitial pneumonia and hard metal exposure.

Methods: Surgical pathology files from 2001 to 2004 were searched for explanted lungs with the histopathologic diagnosis of giant cell interstitial pneumonia, and we reviewed the associated clinical histories. Mass spectrometry, energy-dispersive x-ray analysis, and human leukocyte antigen typing data were evaluated.

Results: Of the 455 lung transplants, 3 met the histologic criteria for giant cell interstitial pneumonia. Patient 1 was a 36-year-old firefighter, patient 2 was a 58-year-old welder, and patient 3 was a 45-year-old environmental inspector. None reported exposure to hard metal or cobalt dust. Patients 1 and 2 received double lung transplants; patient 3 received a left single-lung transplant. Histologically, giant cell interstitial pneumonia presented as chronic interstitial pneumonia with fibrosis, alveolar macrophage accumulation, and multinucleated giant cells of both alveolar macrophage and type 2 cell origin. Energy-dispersive x-ray analysis revealed no cobalt or tungsten particles in samples from the explanted lungs. None of the samples had detectable tungsten levels, and only patient 2 had elevated cobalt levels.

Take home message: compelling argument that GIP is not limited to individuals with hard metal exposure, and other environmental factors may elicit the same histologic reaction.


First report of pneumonitis with this drug.

Interesting case study; patient is still alive 36 months after diagnosis.