Lung Cancer
New Concepts and Practical Considerations for Diagnosis

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Topics

• Atypical adenomatous hyperplasia
• Bronchioloalveolar carcinoma (BAC) = adenocarcinoma in situ (AIS)
• The new IASLC classification of peripheral adenocarcinomas
• Immunohistochemical staining for the classification of small biopsies with carcinoma

Needle core bx of peripheral lung nodule in a cigarette smoker
The correct diagnosis for the images in the last 2 slides is:

• A. Atypical adenomatous hyperplasia (AAH)
• B. Bronchioloalveolar carcinoma (BAC), non-mucinous type (= adenocarcinoma in situ)
• C. Adenocarcinoma, Noguchi type A
• D. Adenocarcinoma, NOS
• E. BAC (adenocarcinoma in situ) vs invasive adenocarcinoma: complete excision required for definitive diagnosis
Introduction: Small peripheral adenocarcinomas

- A tremendous amount of effort has been devoted to finding “early” curable lung cancers
- Criteria for “early” are unclear, since up to 15% of adenocarcinomas <1 cm in diameter are metastatic on presentation (Miller DL. Ann Thoracic Surg 2002 & Yoshida J. Chest 1998)
- Stage 1A (T1aN0, “early”) tumors have a 75% five year survival, implying that there are favorable and unfavorable variants within this category
- Recent studies have suggested that there are favorable and unfavorable prognostic patterns in small peripheral (stage 1A) adenocarcinomas

Abreviations

- AAH = atypical adenomatous hyperplasia
- BAC = bronchioloalveolar carcinoma
- AIS = adenocarcinoma in situ (new name for BAC under the new IASLC classification)
- MIA = minimally invasive carcinoma
- GGO = ground glass opacity

Atypical Adenomatous Hyperplasia

- WHO 2004: –AAH is a localized proliferation of mild to moderately atypical cells lining alveoli and, sometimes, respiratory bronchioles, resulting in focal lesions in peripheral alveolated lung, usually less than 5 mm in diameter, and generally in the absence of underlying [diffuse] interstitial inflammation and fibrosis”
Atypical Adenomatous Hyperplasia

- Synonyms
  - Bronchioloalveolar cell adenoma (Miller 1990)
  - Atypical alveolar cell hyperplasia
  - Atypical alveolar hyperplasia
  - Atypical bronchioloalveolar cell hyperplasia

- The term “atypical adenomatous hyperplasia” is generally used in the current literature.

Atypical Adenomatous Hyperplasia

Prevalence by Underlying Cancer Type*

- Carcinomas metastatic to lung 4-10%
- Squamous cell carcinoma 3-11%
- Adenocarcinoma 16-35%
- Large cell carcinoma 10-25%
- All primary lung ca 9-21%

- Implication: AAH is associated with adenocarcinoma and large cell carcinoma (many of which are poorly differentiated adenocarcinomas)

*WHO 2004

Atypical Adenomatous Hyperplasia

Imaging Findings

- Single or multiple small nodules of ground glass opacity (GGO)
  - GGO indicates opacity that does not obscure underlying lung vessels or airways
- Pure GGO nodules are not specific and are seen with BAC (adenocarcinoma in situ), and with benign processes
Atypical Adenomatous Hyperplasia

**Histologic Features**

- Proliferation of cells with features of Clara cells or type II cells along alveolar walls
- Proliferating cells show variable but generally not marked degrees of cytologic atypia and variable shape including rounded and peg shaped-forms
  - Nuclear pseudo-inclusions common
- **Proliferating cells are generally discontinuous** (as opposed to BAC/AIS)
- Often associated with underlying fibrotic reaction that stops abruptly with the edge of the proliferating lesion
- Usually <5mm in diameter
By size and maybe cytology this should be a BAC, but the cells are spaced which is more the pattern of AAH.

The distinction between AAH and BAC can be arbitrary, but it doesn't matter! If this lesion is completely excised no further therapy is needed.
Atypical Adenomatous Hyperplasia

- Restrictions on diagnosis
  - Ciliated cells are not part of AAH
  - Metaplastic changes secondary to underlying fibrosis are not AAH
  - You cannot diagnose AAH in the presence of diffuse underlying interstitial fibrosis or diffuse interstitial inflammation—AAH is always a discrete lesion
Atypical Adenomatous Hyperplasia

- Genetic changes
  - P53 mutations rare in AAH, more common in BAC, and even more common in adenocarcinomas
  - FISH studies show frequent aneuploidy of chromosome 7 in AAH; percent of aneuploid cells increases from AAH to adenocarcinoma
  - Activating EGFR mutations seen in 1/3 of AAH and most nonmucinous BAC (Sakuna 2007)
  - Some AAH and BAC from the same patient show the same EGFR mutations (Sartori 2008)
  - Some AAH are clonal proliferations
- Implication: AAH are either BAC/adenocarcinoma precursors, or very small BAC

Separation of AAH and BAC/AIS

Yousem and Beasley: Arch Path 2007

- AAH usually <5mm and BAC usually >5mm (but size overlaps occur)
- AAH has a discontinuous row of proliferating cells; BAC has continuous crowded cells
- AAH does not have nuclear stratification, tufting, papillary formations, BAC may have these features
- AAH does not have the degree of cytologic atypia usually seen in BAC
- "Separation of AAH from BAC may be extremely difficult"
Atypical Adenomatous Hyperplasia

- Prognostic significance of AAH
  - AAH are usually multifocal when detected and therefore probably always untreatable surgically
  - Postoperative (post lung ca resection) followup of patients with and without AAH shows no difference in survival (WHO 2004)

- Practical conclusion
  - The finding of AAH in a surgical pathology specimen does not require any further treatment

Bronchioloalveolar Carcinoma (BAC): Definition

- WHO 1999 and 2004: An adenocarcinoma showing “growth of neoplastic cells along pre-existing alveolar structures (lepidic growth), without evidence of stromal, vascular, or pleural invasion.”
- If strictly adhered to, this definition means that BAC is in situ carcinoma and therefore 100% curable

- Under the new proposed IASLC classification, nonmucinous BAC are termed “adenocarcinoma in situ” (AIS)
- NB: Many (?most) respiratory physicians and thoracic surgeons still use “BAC” (more on this later!)
Radiologic Findings in BAC/AIS

- Pure BAC usually appear as a ground glass opacity
- Increased density in the center usually indicates a central scar or invasive component

NB: Many pure GGO are benign inflammatory processes, particularly if small
Morphologic Subtypes of BAC/AIS

• Nonmucinous: Tumor cells are type II or Clara cells with eosinophilic or clear cytoplasm
  – Nuclear pseudoinclusions common
  – Apical snouts (Clara cell differentiation) common
  – Tumor cells show crowding, overlapping, piling up, papillary formation
  – Tumor cells are cytologically atypical

• The vast majority of BAC/AIS are nonmucinous

• BAC are frequently associated with chronic inflammatory infiltrate, fibrosis, amyloid, or osteocartilagenous metaplasia in the underlying alveolar wall

Morphologic Subtypes of BAC/AIS

• Mucinous: Tumor cells contain mucus droplets and resemble intestinal goblet cells or endocervical cells

• The vast majority of what appear to be mucinous BAC are really invasive adenocarcinomas (―mucinous adenocarcinoma‖ under the new proposed IASPC classification)

• The prognosis of ―mucinous BAC‖ is much worse than that of nonmucinous BAC

• On frozen section it is advisable to tell the surgeon that what looks like a mucinous BAC will probably turn out to be invasive and should be treated as if invasive
Differences between Nonmucinous and Mucinous BAC/AIS

- **Immunohistochemistry**
- **Stain**
  - Nonmucinous
  - Mucinous
- **CK7**
  - +
  - +
- **CK20**
  - -
  - +
- **TTF-1**
  - +
  - -
- **Cdx-2**
  - -
  - -

- **Molecular changes**
- **EGFR mutations**
  - Common
  - Uncommon
- **KRAS mutations**
  - Uncommon
  - Common
Morphologic Subtypes of BAC/AIS

- Distinction between mucinous and nonmucinous BAC is important
- Nonmucinous BAC represents adenocarcinoma in situ carcinoma and has a 100% survival rate
- Nonmucinous BAC often responsive to EGFR (epidermal growth factor receptor)-targeted therapy
- Most "mucinous BAC" tend to present with multifocal disease or pneumonic spread and most show invasion somewhere, hence it is preferable to label these as mucinous adenocarcinomas
  - Prognosis much worse than nonmucinous BAC/AIS

Separation of BAC/AIS from Reactive Epithelial Proliferations

- Reactive alveolar lining cells are common in acute and chronic lung processes, especially interstitial pneumonias
  - Diagnosis of BAC/AIS in this setting requires evidence of a mass lesion and proper growth pattern
- Reactive alveolar lining cells sometimes are flattened and clearly arise from flat alveolar epithelial cells; however, they can also be peg shaped or type II shaped, or look like ciliated or Clara cells
  - Carcinomas don’t have ciliated cells
- The diagnosis of BAC/AIS or AAH requires evidence of a mass lesion!
Peribronchiolar metaplasia

Prior to 1999 the diagnosis of BAC was a dog's breakfast—no consistency of definitions or diagnoses among pathologists, and included:

- BAC
- BAC type lesions with small or not so small areas of invasion
- Lesions with extensive invasion but some lepidic growth at periphery

WHO 1999 adopted the definition of BAC as in situ disease because of work from Japan (Noguchi 1995) showing that pure BAC or tumors that were largely BAC with small central scars had an excellent prognosis.

Since 1995 a variety of predictors of prognosis in small peripheral adenocarcinoma have been published.
Growth Pattern Features Suggested to Be Useful in Predicting Prognosis in Small Peripheral Adenocarcinoma

- Pattern of lepidic vs nonlepidic growth (stromal invasion) [Noguchi 1995]
- Size of central scar [Suzuki 2000; Sakurai 2004]
- Pattern of central scar [Maeshima 2002]
- Percentage of lepidic growth [Minami 2005]
- Percentage/presence of papillary growth [Aida 2004]
- Size of invasive area [Yim 2007]
- Size of invasive area [Borzuk 2009]

Classification of Small Peripheral Adenocarcinomas of the Lung

M Noguchi et al: Cancer 1995

- Subtype A: Pure lepidic growth (ie, BAC)
- Subtype B: Lepidic growth with focal "structural collapse" of alveoli [ie, central scar]
- Subtype C: Lepidic growth with "foci of active fibroblastic proliferation" [ie, desmoplastic reaction]
- Subtype D: Poorly differentiated [solid] adenocarcinoma
- Subtype E: Tubular [cribiform] adenocarcinoma
- Subtype F: Papillary adenocarcinoma

Nonmucinous BAC/AIS = Noguchi A
This pattern immediately raises a question of invasion.

Noguchi C: Lepidic growth with invasive area

Looks like Noguchi B ("collapse") here.
Noguchi C Pattern = "active fibroblast proliferation" = desmoplasia

Noguchi D: Lepidic growth with overt carcinoma

Looks like Noguchi B here

But not here
Survival in 174 Small (<2 cm) Peripheral Adenocarcinomas
By Morphologic Subtype (Noguchi: Cancer 1995)
Problems with the Noguchi Classification

- Separating “collapse” from “invasion” often very difficult
  - Central scar may be associated with Noguchi B, C, or D pattern
- Features suggested to represent true invasion
  - Angulated glands
  - Glands have more cytologic atypia than lepidic component
  - Desmoplastic reaction
  - Destruction of elastic tissue and alveolar walls
  - Single cell, cribiform, complex acinar growth
  - (from Yousem & Beasley Arch Path 2007)

Classification of Small Peripheral Adenocarcinomas Using the Central Scar

- Suzuki et al (Ann Thor Surg 2000) suggested that simply measuring the size of the central scar provided the same prognostic information as the more complicated Noguchi grades
- Maeshima et al (Cancer 2002) suggested using density and size of desmoplastic reaction (“scar grade”)
Survival in 100 peripheral adenocarcinomas <5cm in diameter by size of central scar

Suzuki Ann Thor Surg 2000

Histologic Features are Important Prognostic Indicators in Early Stages Lung Adenocarcinoma

Yim et al: Modern Pathology 2007

Note: In this study any pattern in the center of the BAC, including pure scar, was considered “invasion.” Surgical procedure apparently = lobectomy.

Invasive Size is an Independent Predictor of Survival in Pulmonary Adenocarcinoma

Am J Surg Path 2009
Prognosis in 294 Small Peripheral Adenocarcinomas  
(Boland et al: Abstract presented at USCAP 2012)

- 5 Year Survivals:
- BAC (Adenocarcinoma in Situ) 100%
- <5mm Invasion 78%
- >5mm Invasion 60%

Proposed New IASLC/ATS/ERS Classification of Adenocarcinomas  
(Travis et al: J Thoracic Oncol 2011)

| Pure lepidic growth = BAC/AIS | Lepidic growth, <5mm invasion = “Minimally invasive adenocca” |

| Lepidic growth, >5mm invasion = “Lepidic predominant adenocca” |

| TABLE 1. | IASLC/ATS/ERS Classification of Lung Adenocarcinoma in Resection Specimens |

<table>
<thead>
<tr>
<th>Presumptive terms:</th>
<th>Atypical adenomatous hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma in situ (&lt;0.5 cm tumor) BAC</td>
<td></td>
</tr>
<tr>
<td>Nonevasive</td>
<td>Micronodule</td>
</tr>
<tr>
<td>Mixed invasive/extravascular</td>
<td>Invasive adenocarcinoma</td>
</tr>
<tr>
<td>Lepidic predominant (tumor more than 5 mm invasive)</td>
<td></td>
</tr>
<tr>
<td>Acinar predominant</td>
<td></td>
</tr>
<tr>
<td>Papillary predominant</td>
<td></td>
</tr>
<tr>
<td>Micropapillary predominant</td>
<td></td>
</tr>
<tr>
<td>Solitary predominant with raise periphery</td>
<td></td>
</tr>
<tr>
<td>Variants of invasive adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Invasive mucinous adenocarcinoma (tumor more than 5 mm invasive)</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td></td>
</tr>
<tr>
<td>Proliferative (low and high grade)</td>
<td></td>
</tr>
<tr>
<td>Emerio</td>
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</tbody>
</table>

BAC, bronchioalveolar cell carcinoma; IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society. |
Proposed New IASLC/ATS/ERS
Classification of Adenocarcinomas
(Travis et al: J Thoracic Oncol 2011)

• BAC/Adenocarcinoma in Situ
  • For lesions < 3cm in diameter
  • Nonmucinous BAC now classified as "adenocarcinoma in situ" (AIS)
  • Mucinous BAC viewed as invasive mucinous adenocarcinomas (with rare exceptions)
  • NB: The whole lesion must be examined to make these diagnoses. It cannot be done on small biopsies

• Nonmucinous BAC: Now Adenocarcinoma in situ

Proposed New IASLC/ATS/ERS
Classification of Adenocarcinomas
(Travis et al: J Thoracic Oncol 2011)

• Minimally Invasive Adenocarcinoma
  • Only applies to tumors <3cm in diameter
  • Lepidic pattern with <5mm of invasion
  • NB: This diagnosis should not be made if:
    – Tumor invades blood vessels, lymphatics, pleura
    – Necrosis present
  • If multiple separate invasive foci present, measure the largest one
  • The whole tumor must be sampled to make this diagnosis; the dx cannot be made on small biopsies
Pure lepidic growth = BAC/AIS

Lepidic growth, <5mm invasion = "Minimally invasive adenoca"

Lepidic growth, >5mm invasion = "Lepidic predominant adenoca"

11 mm BAC (AIS)?
1mm focus of invasion: Correct Dx: Minimally invasive adenocarcinoma (or Noguchi type C adenocarcinoma or BAC with 1mm of invasive carcinoma)

Proposed New IASLC/ATS/ERS Classification of Adenocarcinomas
(Travis et al: J Thoracic Oncol 2011)

- Invasive Adenocarcinomas
  - Lepidic predominant (nonmucinous BAC pattern with >5mm of invasion)
  - Acinar predominant
  - Papillary predominant
  - Micropapillary predominant
  - Solid predominant with mucin production
  - NB: record all patterns present

Pure lepidic growth = BAC/AIS
Lepidic growth, <5mm invasion = "Minimally invasive adenoca"
Lepidic growth, >5mm invasion = "Lepidic predominant adenoca"
Lung Adenocarcinoma: Prognostic Patterns

From Yoshizawa et al. Mod Path 2011

Low Grade
- BAC (adenocarcinoma in situ)
- Minimally invasive adenocarcinoma (<5mm invasion)

Intermediate Grade
- Lepidic predominant (>5mm invasion)
- Acinar predominant
- Papillary predominant

High Grade
- Micropapillary predominant
- Solid predominant
- Colloid predominant
- Invasive mucinous
Stage 1 Adenocarcinoma: Disease Free Survival by Histologic Pattern

From Yoshizawa et al: Mod Path 2011

Reproducibility of histopathological subtypes and invasion in pulmonary adenocarcinoma. An international interobserver study

26 pathologists reviewed single images of adenocarcinomas

Box Plot of the Dominant Pattern Score

Figure 8 Box plot distribution of the dominant pattern scores. The pattern agreement (draw agreement) is shown for the different patterns and the different subtypes of adenocarcinoma. The box plot indicates the interquartile range, the median, and the full range of the scores. The whiskers represent the range, excluding outliers. Outliers are shown as individual points.
Reproducibility of histopathological subtypes and invasion in pulmonary adenocarcinoma. An international interobserver study

Role of the Pathologist in Dealing with Pure GGO or Partial GGO Nodule on Frozen Section*

- Assuming that there is no prior tissue diagnosis and the surgeon gives you a wedge of lung:
- Confirm on frozen section that this is a malignancy (pure GGO often benign)
- Determine if pure BAC/AIS on frozen section (implies surgery stops with the wedge)
- Determine if obvious adenocarcinoma with >5mm invasion on frozen section (implies surgeon does a completion lobectomy)
- Determine if there is invasion <5mm in extent on frozen section (surgeon may or may not do a completion lobectomy, but most probably will)

*This is what happens at Vancouver General Hospital, but your hospital may be very different!

Conclusions re Proposed IASLC Classification - I

- New classification makes sense in terms of prognosis and eliminates issues of collapse vs invasion
- You must sample the whole lesion to make these diagnoses
  – Including a complete cross section on frozen section
Conclusions re Proposed IASLC Classification -II

• BAC is enshrined in the literature and in the minds of clinicians (pulmonologists, oncologists, thoracic surgeons)
• If you use AIS and other new categories, it’s wise to indicate old and new diagnoses
  – “Adenocarcinoma in situ (Bronchioloalveolar cell carcinoma)”
  – “Minimally invasive adenocarcinoma (BAC with central invasive focus of x mm)”

Conclusions re Proposed IASLC Classification -III

• You need to agree with your thoracic surgeons about how to report these lesions, especially on frozen section
• NB: Most but not all thoracic surgeons believe that BAC (AIS) can be treated by wedge excision
• NB: Many thoracic surgeons will treat any lesion with invasion by lobectomy
• The jury is still out on whether minimally invasive carcinoma behaves as well as AIS

Role of Immunohistochemistry in Classifying Carcinoma of the Lung

? Diagnosis
Role of Immunohistochemistry in Subclassifying Lung Cancers on Small Biopsies
• Sorting out small cell from non-small cell carcinoma
• Separating squamous cell from adenocarcinoma
• Immunohistochemistry most useful when the biopsy is very small or tumor poorly differentiated
• Also useful for sorting out primaries from mets
• If tumor not classifiable by immunochemistry, then the term “no small cell carcinoma” is still appropriate

Why the Pathologic Distinction “Small Cell vs Non-Small Cell Carcinoma” Isn’t Good Enough Any More - I
• WHO 2004 recommended use of “small cell” and “non-small cell lung cancer”
• Most small cell carcinoma treated by chemotherapy/radiation rather than surgery
• Most (70%) adenocarcinomas and squamous cell carcinomas have metastasized at time of presentation and chemotherapy is the only treatment option

Why the Pathologic Distinction “Small Cell vs Non-Small Cell Carcinoma” Isn’t Good Enough Any More - II
• One size no longer fits all
• Increasingly, targeted therapy depends on cell type
• Adenocarcinomas may respond to:
  – EGFR inhibitors (Iressa, Tarceva)
  – Pemetrexed (Alimta)
  – Anti-VEGF therapy (Bevacizumab)
  – Crizotinib for EML4-ALK rearrangement tumors
  – Kras directed therapies may be developed
Why the Pathologic Distinction ―Small Cell vs Non-Small Cell Carcinoma‖ Isn’t Good Enough Any More - III

• Squamous cell carcinomas
  – Typically do not show EGFR or kRAS mutations, thus no point to using EGFR inhibitors
  – Do not respond to pemetrexed (Alimta)
  – Squamous cell carcinomas may bleed massively with anti-VEGF therapy (Bevacizumab)
  – New targeted therapies for squamous cell coming
    • FGFR1 inhibitors for tumors with FGFR1 amplification (Sci Trans Med 2010)

Role of Immunohistochemistry in Subclassifying Primary Lung Cancers: Potentially Useful Markers

• Squamous  Adeno Small Cell
• p63+ TTF-1+   TTF-1+
• CK5/6+ p63- Chromogranin+
• TTF-1- CK5/6- Synaptophysin+
• CK7- CK7+ CD56+
• Napsin- Napsin + Napsin-

225 adenocarcinomas and 200 squamous cell carcinomas tested in tissue microarrays to simulate small biopsies
Panel tested: p63, TTF1, CK5/6, 34bE12, Napsin A, Mucicarmine, NTRK1, NTRK2 (both squamous cell markers)
Criteria for positivity: >1% staining except TTF-1 >10% staining
Conclusions from Terry et al

- Single best marker = p63 (sensitivity 84%, specificity 85%)
- Most efficient panel in terms of tissue preservation: p63, TTF-1, CK5/6, CK7 (sensitivity and specificity >90%) (my conclusion)
- Authors suggest doing stepwise staining; however, multiply recutting blocks loses tissue (my conclusion)
39 poorly differentiated carcinomas on biopsy compared with subsequent resection specimens
>10% of cells staining called positive

In 28 of 39 cases (72%) staining identical across 6 antibodies in biopsy and resection specimens

In 10 cases 1 antibody was discrepant/In 1 case 2 antibodies were discrepant

1 misdiagnosed case (adenocarcinoma TTF-1/Napsin A negative on biopsy, positive on resection) = 2.5% misdiagnosed

**TABLE 2. Sensitivity and Specificity of Immunohistochemical Markers for Adenocarcinoma in Lung Biopsies**

<table>
<thead>
<tr>
<th>Markers</th>
<th>TTF-1</th>
<th>Napsin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>11.19</td>
<td>11.19</td>
</tr>
<tr>
<td>Specificity</td>
<td>15.17</td>
<td>15.17</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>24.24</td>
<td>24.24</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>12.24</td>
<td>12.24</td>
</tr>
</tbody>
</table>

Napsin A is specific but doesn't add much because it's insensitive; CK7 is the opposite (Churg)

**TABLE 3. Sensitivity and Specificity of Immunohistochemical Markers for Squamous Cell Carcinoma in Lung Biopsies**

<table>
<thead>
<tr>
<th>Markers</th>
<th>p63</th>
<th>CK5/6</th>
<th>34E12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>11.15</td>
<td>11.15</td>
<td>11.15</td>
</tr>
<tr>
<td>Specificity</td>
<td>22.24</td>
<td>22.24</td>
<td>22.24</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>24.24</td>
<td>24.24</td>
<td>24.24</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>12.24</td>
<td>12.24</td>
<td>12.24</td>
</tr>
</tbody>
</table>

p63 has good sensitivity and specificity; CK5/6 has good specificity but only moderate sensitivity (Churg)
Conclusions from Mukhopadhyay & Katzenstein

**Whithaus’ Conclusions**

- For adenocarcinoma
  - Napsin: 83% sensitivity, 98% specificity
  - TTF-1: 60% sensitivity, 98% specificity
- For squamous cell carcinoma
  - CK5: 53% sensitivity, 96% specificity
  - p63: 95% sensitivity, 86% specificity
- They recommend panel of Napsin A + p63
  - Sensitivity 94%
  - Specificity 96%
N=315 retrospective cases
N=38 prospective biopsies followed by resections

Heatmap of Staining Reactions

Single Marker Performance for Separating Adeno from Squamous Cell Carcinoma

Diffuse TTF-1, p63, or CK5/6 staining is diagnostic (Churg)
Rekhtman’s Conclusions

- Some reactivity for squamous’ markers (p63, CK5/6) is common in adenocarcinoma, but diffuse reactivity is not
- Reactivity for TTF-1 is rare in squamous carcinomas and is never diffuse
- Combination of TTF-1/p63 staining classifies the majority of tumors as adeno vs squamous cell ca
- Addition of CK5/6 will accurately classify almost all the rest

Modern Pathology 2012; 25:405-415

p40 (Δp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma

A Study of Δp63 Expression in Lung Non-Small Cell Carcinomas

Am J Surg Pathol 2012; 36: 895-899

Anti-p40 (p63 splice variant) has the same specificity as anti-p63 for pulmonary squamous cell carcinomas, but has a very low positivity rate (<5%) for lung adenocarcinomas

Churg’s Real-World Conclusions: Adeno vs Squamous Cell Carcinoma - I

- **Diffuse** p63 and/or CK5/6 = squamous cell
  - p63 and CK5/6 are positive in a proportion of adenocarcinomas, but not diffusely positive
- The combination of p63 and CK5/6 positive staining virtually eliminates adenocarcinoma
- **Diffuse** TTF-1 staining = adenocarcinoma
  - TTF-1 is positive in a small proportion of squamous cell carcinomas but not diffusely positive
- Whether Napsin is better or worse than TTF-1 is controversial: unclear at this point whether it is worth adding
Poorly differentiated carcinoma on bx

Features suggestive of squamous cell

Tumor is p63 and CK5/6 +
Confirms diagnosis of squamous cell ca
Poorly differentiated carcinoma? dx

Correct dx: poorly differentiated adenocarcinoma
Poor differentiated tumor on bx

p63

CK5/6

TTF-1

Dx = poorly differentiated adeno (don’t diagnose large cell on biopsy)
**Diagnosis**

- p63
- CK5/6
- TTF-1
- Entrapped lung

- Poorly differentiated squamous cell
Fly-in-Ointment: Interobserver Agreement: Squamous vs Nonsquamous Cell
Grilley-Olson et al: Arch Path 2012
- Panel of 12 expert lung pathologists and 12 community pathologists
- Each reviewed digital image of a single H&E stained slide of 96 resected lung cancers
- Kappa coefficient 0.40 for community pathologists, 0.65 for expert pathologists
- Implications (Churg):
  - We are now assigning poorly differentiated tumors to squamous vs non-squamous by IHC, because expert pathologists can't do it by H&E
  - The clinical significance of doing this is unclear

Some Other Staining Confounders
- TTF-1 staining
  - Normal entrapped lung (Napsin does this also)
  - Small cell ca of lung
  - Metastatic thyroid carcinomas
  - Occasional breast and gyn tumors
  - Solution: Add Napsin A
    - Some papillary renal cell ca stain with Napsin
- Strong TTF-1 and p63
  - Consider adenosquamous carcinoma
- Strong p63
  - Metastatic squamous cell, urothelial, occ lymphomas and sarcomas

Churg’s Real-World Conclusions: Adeno vs Squamous Cell Carcinoma - I
- Diffuse p63 and/or CK5/6 = squamous cell
  - p63 and CK5/6 are positive in a proportion of adenocarcinomas, but not diffusely positive
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  - TTF-1 is positive in a small proportion of squamous cell carcinomas but not diffusely positive
  - Whether Napsin is better or worse than TTF-1 is controversial; unclear at this point whether it is worth adding
Churg’s Real-World Conclusions: Adeno vs Squamous Cell Carcinoma - II

- Sensitivity & specificity in these studies probably exceed realistic underlying diagnostic accuracy!
  - Interobserver agreement is poor on poorly differentiated carcinomas and poorly differentiated carcinomas are the problem
- Run TTF-1/p63/CK5/6/ and maybe Napsin A or CK7
- Do it only once! Avoid recutting blocks multiple times to preserve tissue for molecular studies
- Do it only once! It’s more efficient in terms of reporting

Churg’s Real-World Conclusions: Adeno vs Squamous Cell Carcinoma - III

- Don’t forget that TTF-1 is positive in almost all small cell carcinomas
- The preceding algorithm only works if you are sure that you are not dealing with a small cell carcinoma!
  - Napsin may be helpful if you are not sure

Useful Markers For Small Cell Ca
(From PathIQ-Immunquery)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Range of Positive %</th>
<th>N</th>
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</thead>
<tbody>
<tr>
<td>CD56</td>
<td>91-98</td>
<td>143</td>
</tr>
<tr>
<td>TTF-1</td>
<td>76-84</td>
<td>430</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>44-54</td>
<td>397</td>
</tr>
<tr>
<td>Chromogranin</td>
<td>37-45</td>
<td>548</td>
</tr>
<tr>
<td>AE1/AE3</td>
<td>77-91</td>
<td>104</td>
</tr>
</tbody>
</table>
Value of CD56 in Small Cell Ca  
(Kaufmann, Human Path 1997)

- \( N = 70 \) small cell, 344 non-small cell ca
- CD56 sensitivity 94% and specificity 95% for small cell ca (of any site, not only lung)
  - Staining should be membranous
  - Occasional non-small cell carcinomas stain
- Neuroendocrine markers only had a sensitivity of 44%

Value of CD56 in Small Cell Ca  
(Churg)

- CD56 also stains some lymphomas (morphologic confounder)
- Use it with TTF-1 and/or neuroendocrine markers for diagnosis small cell ca of lung
Immunohistochemical staining was not incorporated as a required core element, but only because we wanted to make the criteria applicable to locations where immunohistochemical staining is not available.

Do you need to stain every case?

- H&E is still the gold standard!
- There seems to be no good reason to stain cases that are obviously squamous cell or adeno on H&E
  - But we may be forced into doing so (by analogy with the situation in lymphomas)
- Small cell ca should probably be confirmed by IHC
- Large cell carcinoma should not be diagnosed on biopsy
  - If IHC doesn’t help, S/O as poorly differentiated non-small cell