Respiratory cytology, EBUS and ancillary tests

Lukas Bubendorf
• Technique
• Normal findings
• Non-neoplastic changes
• Bronchoalveolar lavage

• Tumors
• Predictive marker analyses
• Sputum
• Bronchial secretion
• Brush cytology
• Bronchoalv. lavage (BAL)

• Transbronchial FNA (TBNA)
• Endobronchial US (EBUS)
Bronchoscope

Trachea

Left primary broncus

Unusual tissue sampled for biopsy

Right bronchus

Left bronchus

Secretions
Endobronchial ultrasound (EBUS) FNA
EBUS-FNA

- Mediastinal lymph node staging
- FNA of primary tumor
- High sensitivity
- ROSE (rapid on-site evaluation)
- Cell block
Basal cells
Creola-bodies
Curschmann spirals
Asbestos-bodies
(„ferruginous bodies“)
Bronchoalveolar Lavage (BAL)
0.9% NaCl

Bronchoscope

Three-way cock

3x50ml

middle lobe

Collecting vessel
BAL: Indications

- Diffuse interstitial and alveolar lung diseases
  - E.g. sarcoidosis, hypersens. pneumonitis
- Infections (immunosuppression)
  - E.g. pneumocystis, aspergillus, CMV
- Tumor
  - Bronchioloalveolar carcinoma
Clinical information

- Smoker yes/no
- Occupation
- Immunosuppression yes/no
- HIV status
- i.v. drug abuse yes/no
- Technical course / cough
- Instilled/recovered fluid volume
Hypersensitivity Pneumonitis

T4/Ly

T4/T8 < 0.5

T4-Ly

T8-Ly
Sarcoidosis

T4/T8 > 2.5
• Sarcoidosis
• Tbc
• Wegener

• Hypersensitivity (exogeneous, drugs)
• HIV
• Immunosuppression
• Organizing pneumonia (COP)
Charcot-Leyden crystals

Asthma bronchiale
DD Eosinophilia

- Eosinophilic pneumonia ++(+)
- Parasites (e.g. Ascaris) ++(+)
- Allerg. bronchopulm. aspergillosis ++
- Allergic asthma ++
- Drugs +
- Churg Strauss syndrome ++(+)


Lamellar bodies
(amiodarone-effect)

Lung toxicity in 5-10%
Pneumocystis
Fluorescence for rapid detection of infectious agents in BAL specimens

- Result within 1.5 hours
- Immunofluorescence (commercial assays)
  - Pneumocystis, Legionella, CMV, RSV, Adenovirus
- Fluorescence stains
  - Auramin-Rhodamin: Acid-fast bacteria
  - Fungiqual A: Funghi
Auramin
„Atypical alveolar proliferation“
„Atypical alveolar proliferation“

- Diffuse alveolar damage (ARDS)
- Drugs (cytostatic)
- Lung infarction
- others
- DD: Adenocarcinoma (bronchioloalveolar)
Chemotherapy (BMT)
Equivocal lung cytology in ~ 2% of the lung patients

138/6’890 (BS 1999-2003)
Multi-probe FISH in lung cytology

LAVysion™ (Vysis, Inc)

5p15  6  7p12 (EGFR)  8q24 (MYC)

normal

CA
Automated relocalization
FISH in equivocal lung cytology (n=45)

Sensitivity: 79%
Specificity: 100%
PPV: 100%
NPV: 74%

Savic S, Chest 2006
2004 WHO Classification of Lung Cancer

Squamous cell carcinoma
- Papillary
- Clear cell
- Small cell
- Basaloid

Adenocarcinoma
- AC, mixed subtype
- Acinar AC
- Papillary AC
- Bronchoalveolar carcinoma
  - Nonmucinous
  - Mucinous
  - Mixed nonmuc. and muc.
- Solid Adenocarcinoma with mucin production
  - Fetal AC
  - Mucinous carcinoma
  - Signet ring adenocarcinoma
  - Clear cell adenocarcinoma

Small Cell Carcinoma

Large cell carcinoma
- Large cell neuroendocrine carcinoma
- Basaloid carcinoma
- Lymphoepithelioma-like carcinoma
- Clear cell carcinoma
- LC carcinoma with rhabdoid phenotype
Bronchial secretion

Brush cytology
Bronchioloalveolar pattern
Nuclear polymorphy
High nuclear/cytoplasmic ratio

nuclear moulding
No prominent nucleoli
# Neuroendocrine tumors of the lung

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Necrosis</th>
<th>Mitoses/10HPF (2mm²)</th>
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</thead>
<tbody>
<tr>
<td>Carcinoid</td>
<td>-</td>
<td>&lt;2</td>
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<tr>
<td>Atyp. carcinoid</td>
<td>+</td>
<td>2-10</td>
</tr>
<tr>
<td>Large cell neuro-endocrine LC</td>
<td>++</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>+++</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Feature</td>
<td>SCLC</td>
<td>LCNELC</td>
</tr>
<tr>
<td>------------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Ncl size</td>
<td>3xEry</td>
<td>&gt;4xEry</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>+/-</td>
<td>+++</td>
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<tr>
<td>Cytoplasm</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Ncl/cytopl.</td>
<td>+++</td>
<td>lower</td>
</tr>
</tbody>
</table>
„Pepper-and-salt“
Questions to the cytopathologist

- Malignancy YES/NO
- SCLC vs. NSCLC
- Primary vs. metastasis
- NSCLC subtype
- Predictive markers
Diagnostic limitations of small biopsies / cytology

- Most tumors (70-80%) not resected
- Small size of biopsies / cytology (40%)
- Classification defined by morphology
- WHO classification based on resection specimens
WHO Classification of Lung Cancer

-2009

Non-Small Cell Lung Cancer (NSCLC)

Small Cell Lung Cancer
## WHO Classification of Lung Carcinomas (2011)

<table>
<thead>
<tr>
<th>NSCLC</th>
<th>Small cell carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>others (rare)</td>
<td></td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>&lt;20%</td>
</tr>
</tbody>
</table>
Advanced NSCLC: subtype matters

• **Predictive of response**
  – EGFR mutation (AC) TKI’s
  – AC vs. non-AC pemetrexed
  – EML4-ALK fusion ALK inhibitor

• **Predictive of toxicity**
  – Bevacizumab contraindicated in SqCC

Travis WD et al, *JTO* 2011;6:244-85
Diagnosis of NSCLC in bronchial biopsies and cytologies

- Glandular structures or mucus, typical cytology → AC
- Unequivocal squamous Differentiation (Keratinization, intercellular bridges) → SCC
- All others: → NSCLC-NOS
The menu of IHC markers (AC vs. SqCC)

- TTF1
- Napsin
- Surfactant A/B
- CK7

- CK5/6
- p63
- Desmocollin-3

Adenocarcinoma

Squamous cell carcinoma
Subtyping of NSCLC-NOS in FNA 4-antibody panel (cell blocks; n=103)

Napsin

p63

TTF1

Desmocollin3

Righi L et al, Cancer 2011; Epub
Predictive marker analysis in non-squamous NSCLC
Predictive markers

Non-squamous → Pemetrexed +

EGFR mutation → TKI +

EML4-ALK rearrangement → ALK inhibitor
Histology or Cytology?
≥30 cells → DNA extraction → PCR amplification → sequencing
Laser Microdissection „Laser Pressure Catapulting“ (PALM®)

At least 30-50 tumor cells
Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

A Percent Change in Tumor Burden

- Disease progression
- Stable disease
- Partial response
- Complete response

Patient No.
Positive: deletion

Positive: break-apart (inversion)

3' ALK 5' ~ 12 Mb EML4

3' ALK EML4 5'
EML4-ALK fusion in NSCLC

- 3 - 13%
- Basel: 4/23 tested NSCLC (17%)
- Heterogeneous (50-100% of tumor cells)
- Depends on selection criteria
  - TTF+ adenocarcinomas (Subtypes?)
  - Mainly light/never smoker
  - EGFR/KRAS negative
  - Younger age, men > women?
**Biopsy/Cytology**

- Squamous cell carcinoma
  - no testing
- Adenocarcina
  - AC phenotype
    - TTF1+, CK7+, CK5/6+, p63+
  - SCC phenotype
    - CK5/6+, p63+, CK7-, TTF1-

**EGFR / KRAS**
(Mutation analysis)

- (simultaneously or sequentially)
- negative

**ALK**
(FISH)

- negative

**BRAF/HER2neu**
(Mutation analysis)

- (not yet established)

Jan 2011, Swiss Lung Pathology Working Group
Summary

• Respiratory cytology is a large field

• Infections, interstitial changes, tumors

• Technical issues

• New challenges (predictive medicine)
Thank you
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Common pitfalls in lung cytology

- Reactive changes / regeneration of bronchial epithelium
- “Atypical alveolar proliferation”
  - (e.g. cytostatic drugs, diffuse alveolar damage)
- Lung infarction (macrophages)

NON-SMALL CELL LUNG CARCINOMAS (NSCLC)

- Squamous cell carcinoma (SCC) 30-40%
- Adenocarcinoma (AC) 40-60%
- Large cell carcinoma ~ 10%
- Adenosquamous carcinoma ~ 1-2%
- Sarcomatoid carcinoma ~ 1-2%

Neuroendocrine tumors
<table>
<thead>
<tr>
<th>Cell type</th>
<th>%</th>
<th>x10⁶/L</th>
</tr>
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<tbody>
<tr>
<td>Whole cell count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-smoker</td>
<td>95%</td>
<td>40-100</td>
</tr>
<tr>
<td>smoker</td>
<td>80-90%</td>
<td>100-300</td>
</tr>
<tr>
<td>Makrophages</td>
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<td></td>
</tr>
<tr>
<td>non-smoker</td>
<td>95%</td>
<td>40-100</td>
</tr>
<tr>
<td>smoker</td>
<td>80-90%</td>
<td>100-300</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>&lt;10%</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Neutr. Granulocytes</td>
<td>&lt;10%</td>
<td>&lt;10</td>
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<tr>
<td>Eos. Granulocytes</td>
<td>&lt;0.5%</td>
<td>&lt;0.5</td>
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<tr>
<td>Mast cells</td>
<td></td>
<td>3/10 HPF</td>
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