Pathobasic

Non-neoplastic Lung Disease I
Program

• Introduction to interstitial lung disease (ILD)/diffuse parenchymal lung disease (DLD)

• Respiratory specimens
  – Overview and handling

• Bronchoalveolar lavage (BAL)
  – Indications
  – Evaluation

• Examples
ILD/DLD

- Spectrum of non-neoplastic inflammatory conditions that share the common property of diffuse involvement of the lung parenchyma
- Acute, subacute and chronic
- Idiopathic or non-idiopathic
  - Most chronic ILD idiopathic (80%)
    → exclude known causes
Multidisciplinary Discussion
Multidisciplinary Discussion

Clinical context + CT pattern

- Bronchoscopy: BAL, biopsy
  - non diagnostic
  - UHB: 20%

- Surgical lung biopsy

Diagnosis, Treatment

adapted from Raj R, Chest, 2016
Histological Specimens

• Fixation
  – for microscopy and molecular analyses: In 10% neutral buffered formalin
    • gentle agitation helps to inflate TBB
  – for electron microscopy (prim. ciliary dyskinesia): In glutaraldehyde

• Microbiology

Leitlinien Makroskopie
http://sgpath.ch/qualitaetssicherung/
Cytological Specimens

• Bronchial brushings, bronchial secretions, (EBUS)-TBNA
  – Smear preparation and immediate Fixation: *alcohol based* (no formalin)
  – Or send material in normal saline

• Bronchoalveolar lavage (BAL)
BAL

- 3x50ml aliquots of normal sterile saline instilled and retrieved

- Sampling:
  - DLD: Lingula
  - Affected area
## BAL Request Form

- **Schnelluntersuchung (nach Tel. Vereinbarung)**
  - Tel.

- **Eilige Untersuchung (am selben Tag)**
  - Tel.

**Klinische Diagnose / Bemerkungen / Fragestellung (bei Studien auch Bezeichnung der Studien)**

### Bronchoalveoläre Lavage

- Instillierte Flüssigkeit..........................ml
- Flüssigkeitsrückgewinn..........................ml
- Raucher pack/year..............................ml
- Immunsupression / wegen........................ml
- i.v Drogenabusus................................ml
- Erreger: PCP  CMV  TBC  RSV  Legionella  Pilze  Adenovirus  H/S-Quotient

- Technisch einwandfrei
- Nichtraucher
- HIV positiv
- Sonstiges

**Datum der Entnahme:**

**Stempel, Unterschrift, Tel-Nr und Fax des eisenden Arztes / Spital**
BAL Indications

• Infections
  – immunocompromised patients
  – persistent pulmonary infiltrates and inadequate response to treatment

• ILD/DLD
  – BAL diagnostic:
    • Pulmonary histiocytosis, eosinophilic pneumonia
  – BAL helpful:
    • Sarkoidosis, HP/EAA, UIP…

• Tumors (lepidic pred. ADC)
Suspected pulmonary infection

Balloon aspiration liquid (BAL)

Microbiology
- Culture: Bacteria, Viruses, Fungi
  - weeks
- PCR: Bacteria, Viruses, Fungi
  - 24-48h

Cytopathology
- Cytomorphology: MGG, PAP, Iron, Fungiquaal A
  - 1-2h
- Fluorescence: IF, Rhodamin-Auramin
  - 1h
Immunofluorescence

1. Incubation with fluorescein labeled antibody
2. Washing
3. Fluorescence microscopy
Detection of Microorganisms

- **Immunofluorescence:**
  - Pneumocystis jirovecii
  - CMV
  - RSV
  - Legionella Pneumophila

- **Fluorescence stainings:**
  - Auramin-Rhodamin: Acid-fast bacteria
  - Fungiqual A: Fungi
Pneumocystis jirovecii

• Nonfilamentous fungi
  – Colonization in hosts with intact immune system
  – Lethal pneumonia in immunocompromised hosts

• Cannot be cultured
  → Goldstandard: Direct detection in BAL

• Three development forms
  – Trophocoit (1-5μm): Immunofluorescence
  – Precyst (5-8μm)
  – Cyst (~8μm): Conventional stains

  → Trophocoit forms 10x more frequent than cysts
Stains used to detect P. jirovecii

PAP

MGG

IF

Grocott
Detection of *Pneumocystis jirovecii* test performance

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tbody>
<tr>
<td>Conventional stains</td>
<td>40-80%</td>
<td>60-99%</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>90-98%</td>
<td>94-100%</td>
</tr>
</tbody>
</table>
CMV

- Latent infection
  - reactivation by immunosuppression
  - reactivation (PCR, Kultur) ≠ CMV-disease

- Cytopathic effect
  - present in 10-20% of culture positive BAL
  - good correlation with CMV pneumonia

- Detection rate doubled by use of monoclonal antibody techniques (IF):
  - positive results in 20-40% of culture positive BAL
  - good correlation with CMV pneumonia
Newly diagnosed HIV infection
Double Infection

Pneumocystis jirovecii  CMV
Immunosuppressed patient under chemotherapy
Double Infection

Pneumocystis jirovecii

CMV
Pulmonary Aspergillosis

1. **Colonisation**
   - Aspergilloma (single)

2. **Allergic Aspergillosis**
   - Allergic bronchopulmonary Aspergillosis (ABPA)
   - Hypersensitivity Pneumonitis

3. **Invasive Aspergillosis**
   - Seminvasive: Granulomatous, multicystic
   - Angioinvasive
     - Hematolog. disease (after BM-transplantation: ~15%)
     - Blood cultures typically negative, in der BAL ~50%
Pulmonary Aspergillosis

• Ubiquatous mold
  – Inhalation of the spores

• Filamentous fungus with characteristic hyphae
  – Uniform 3-6μm in width
  – Parallel cell walls
  – True septa at regular intervals
  – Branching: 45°, regular, dichotom
  – DD: Fusarium, Scedosporium Spezies
    → mikrobiologic culture
33y, BM-transplantated
Cave: Degenerative changes → Swollen hyphae

- Hyphae -15µm in width
- No parallel cell walls
- Cell walls often collapsed and drilled
Pulmonary infections in immunocompromised patients

- Often atypical clinical presentation
- Double infections common
- Rapid microorganism detection crucial
  – IF in BAL
BAL in ILD/DLD

BAL

- Eosinophils $\geq 25%$
  - Eosinophilic Pneumonia

- Lymphocytes $\geq 50%$
  - Consider Drug Reaction, Acute HP

- Bloody lavage (persists and/or increases on sequential aliquots)
  - Diffuse Alveolar Hemorrhage

- Other findings
  - Specific Diagnosis
  - Non-diagnostic
Total Cell Count

Neubauer hemocytometer (C-CHIP oneway)

Trypan blue (vital stain)
CD4/CD8 ratio

- Normal range: 1-2
- Immunocytochemistry or
- FACS

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lymphocytes</th>
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<tbody>
<tr>
<td>CD3</td>
<td>pan-T</td>
</tr>
<tr>
<td>CD4</td>
<td>T-helper</td>
</tr>
<tr>
<td>CD8</td>
<td>T-suppressor</td>
</tr>
<tr>
<td>CD19</td>
<td>B</td>
</tr>
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</table>
• 80-90% makrophages

• <10 makrophages/HPF
• > ciliates / squamous cells
• degenerated cells
# BAL Differential Cell Count: Normal

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>x10⁶/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cell count</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>50-100</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>-300</td>
<td></td>
</tr>
<tr>
<td><strong>Makrophages</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>&gt;90</td>
<td>40-100</td>
</tr>
<tr>
<td>Smoker</td>
<td>&gt;90</td>
<td>100-300</td>
</tr>
<tr>
<td><strong>Lymphocytes</strong></td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td>&lt;0,5</td>
<td>&lt;0,5</td>
</tr>
<tr>
<td><strong>Mast cells</strong></td>
<td></td>
<td>3/10HPF</td>
</tr>
</tbody>
</table>

*Bubendorf L et. (2011) Pathologie. Zytopathologie; Springer-Verlag*
BAL Evaluation

- Cellular patterns
  - neutrophilic, lymphocytic, eosinophilic
  - mixed
  - predominance of smoking-related Macrophages

- Alveolar macrophages
  - iron
  - foamy cell change

- Microorganisms

- Tumor cells

- Foreign material
BAL as diagnostic tool in ILD/DLD

• Provides useful diagnostic information in patients with suspected ILD/DLD when used in conjunction with comprehensive clinical information and thoracic imaging

• Inflammatory cellular pattern helps to narrow the DD, even though the patterns are nonspecific

• Can provide specific diagnosis
  – infections, some DLDs, malignancies

Meyer KC et al. Am J Respir Crit Care Med 2012
Patient 1
Male with suspicion of sarcoidosis

- BAL
- TBNA mediastinal lymph node
- Bronchial biopsy
**BAL**

Instilled fluid: 200 ml, retrieved fluid: 120 ml

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>10^6/L</th>
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<tr>
<td>Total cell count</td>
<td>91.07</td>
<td></td>
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<tr>
<td>Makrophages</td>
<td>65</td>
<td>59.19</td>
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<tr>
<td>Lymphocytes</td>
<td>34</td>
<td>30.96</td>
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<tr>
<td>Neutrophils</td>
<td>1</td>
<td>0.91</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>0</td>
<td></td>
</tr>
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CD4/CD8 ratio = **18.3**
Sarcoidosis
Diagnostic criteria

1. Compatible clinical and/or radiological picture

2. Demonstration of noncaseating granulomas
   - Biopsy (80%), TBNA (73%), EBUS (96%)

3. Exclusion of other diseases capable of producing granulomas or a similar clinical picture
   - DD: infections (Tbc), HP, drug-induced, sarcoid-like reaction in cancers and lymphomas, berylliosis

ATS, ERS and WASOG. Am J Respir Crit Care Med (160) 1999
Valeyre A et al. Lancet (383) 2014
Sarcoidosis
BAL supports diagnosis

• Total cell count =/↑

• In 90%: Lympocytotis ↑↑ (20-50%)
  – Even when imaging studies are normal
  – DD: HP, NSIP, OP, drug reactions, infections, lymphoma

• Usually «claen» background:
  – Normal neutrophiles, eosinophiles, mast cell counts
  – No plasma cells and no foamy alvolar macrophages

Costabel U. et al. Semin Respir Crit Care Med (31) 2010
Sarcoidosis
BAL supports diagnosis

- In 55%: CD4/CD8↑
  - High variability

- In 15% <1.0

- >3.5 (normal range:1-2):
  - Spec. 93-96%, Sens. 53-59%, PPV 75-94%, NPV 71-85%

Costabel U. et al. Semin Respir Crit Care Med (31) 2010
CD4/CD8 ↑

- Sarkoidosis
- Chronic HP/ EAA
- Infections (Tbc)
- Drugs
  - Methotrexat
  - Ampicillin
  - Nitrofurantoin
  - Sirolimus
- Age
- Langerhans cell histiocyrosis
- IPF

CD4/CD8 ↓

- HP
  - EAA / drug induced
- OP
- Smoking
- AIDS
Patient 2
Female, lymphadenopathy, fever
**BAL**

Instilled fluid: 150 ml, retrieved fluid: 60 ml

<table>
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<tr>
<td>Makrophages</td>
<td>31</td>
<td>12.3</td>
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<tr>
<td>Lymphocytes</td>
<td>64</td>
<td>25.4</td>
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<tr>
<td>Neutrophils</td>
<td>5</td>
<td>1.9</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

CD4/CD8 ratio = 7.6
Auramin-Rhodamin

Mycobacteria
Patient 3
Male, Daptomycin (Cubicin®) for 5 weeks
33% eosinphils
Eosinophilic Pneumonia

• Daptomycin induced eosinophilic Pneumonia

• Eosinophilic pneumonia DD:
  – Secondary
    • Infections (parasitic, fungal)
    • Drug-induced
    • Immunologic or systemic diseases
      – Asthma, ABPA, CVD, Eos. granulomatosis with polyangitis, HIV
  – Idiopathic
Patient 4
Female, caugh, mold exposure, EAA?
<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>$\times 10^6/L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count</td>
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<td></td>
</tr>
<tr>
<td>Makrophages</td>
<td>75</td>
<td>577.5</td>
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<tr>
<td>Lymphocytes</td>
<td>11</td>
<td>84.7</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>14</td>
<td>107.8</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mast cells</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Predominance of Smoking-related Macrophages

Smoking-related ILD:

• RBILD

• Langerhans cell histiocytosis

• DIP

Meyer KC et al. Am J Respir Crit Care Med (9) 2012
Pulmonary Langerhans Cell Histiocytosis

- >90% occur in smokers
- 20-50 years of age
- m=f
- 75% symptomatic
  - dry cough, dyspnoe, fever, weight loss, night sweats
  - 10-20%: spontaneous pneumothorax
- Smoking cessation
- Few progress to lung fibrosis