Lung Biopsy: Who, What, When, Where, Why, and How to interpret the results

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Disclosures

No relevant financial discloses related to content of this presentation.

I am **NOT** a pathologist!

Presentation will be a bit surfactant, ChILD – centric, but overall points are generalizable.
Learning Objectives

• Discuss indications for lung biopsy in newborns, infants & older children.

• Discuss advantages and risks of biopsy compared to other diagnostic modalities.

• Understand the importance of electron microscopy and frozen tissue when performing lung biopsies in children.
Good communication between:
Clinician: – what is the question?
Imager: where to biopsy?
Surgeon: Where, how to obtain, what?
Pathologist: What is the question, how best to use, prepare.
Lung Biopsy: Who?

Lung Transplant Surveillance
  Transbronchial
Establish or confirm diagnosis (or exclude some)
Diagnosis presumed, but atypical features
Results will alter management, inform prognosis
  Opportunistic or unusual infection
Developmental Disorder
Childhood Diffuse Lung Disease – specific diagnosis
  Pulmonary Hemorrhage
  Pulmonary Hypertension, PCH, PVOD
Lung involvement in systemic disease
Conditions diagnosable without lung biopsy

Neuroendocrine Hyperplasia of Infancy
  Typical clinical
  Characteristic HRCT

Autoimmune Pulmonary Alveolar Proteinosis
  Anti-GM-CSF antibodies
  BALF appearance and cytology

Genetic
  CF: Sweat test, genetic
  PCD: Ciliary biopsy, exhaled NO, NGS panels

Surfactant Function Disorders
  Hereditary PAP
  Others: FLNA, COPA, SAVI (TMEM 173), MARS, ITGA3
  PCH, PVOD, PAH
What: Handling of Biopsy Tissue

Cultures

EM Fixative

Frozen

Histology

Langston et al., *Pediatric and Developmental Pathology* 9, 173-180, 2006

Figure 1. This diagrammatic representation of the protocol for handling lung biopsies shows the biopsy centrally with parts A and D (the cut-off staple line) sent for culture; part B is divided for viral culture (B-1), freezing in a bullet or capsule (B-2), and fixation in glutaraldehyde for electron microscopy (B-3); part C is then used to make imprints. Following this, a small section (C-1) is cut off and expanded with cryomatrix/sucrose. The remainder (C-2) is expanded by transpleural formalin instillation and then divided for processing for histologic examination.
# Lung Biopsy: Specimen preparation

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron Microscopy</td>
<td>Glutaraldehyde-Fixed Tissue (1 mm pieces)</td>
</tr>
<tr>
<td>Microbiology Touch Imprint Slides</td>
<td>Sterile Tissue for Cultures (Bacterial, Viral, Fungal, Acid Fast) Slides for Special Stains and Rapid Detection of Organisms</td>
</tr>
<tr>
<td>Histology</td>
<td>Remove Staple Line, Inflated With Formalin, Fix 15–20 minutes, Section Perpendicular to Margin (at least 1/2 of Biopsy)</td>
</tr>
<tr>
<td>Molecular Study</td>
<td>Freeze and Retain at -80°C for PCR, if needed.</td>
</tr>
<tr>
<td>Immunofluorescence Study</td>
<td>Inflated With 1:1 Mixture of Cryo,tric Sucrose Solution. Freeze and Retain for IF, if needed.</td>
</tr>
</tbody>
</table>


**Importance of inflation**

Langston et al., *Ped & Dev Path* 9, 173-180, 2006
Electron Microscopy

Control

SP-B Deficient

ABCA3 Deficient
Utility of Frozen Tissue: Sorting out VUS

Hx: 2 m/o, tachypnea, hypoxemia, FTT

Genetic testing: Heterozygous *SFTPC* c.435 G > A (p.Gln145=)

Normal SP-C Transcript

Sequence transcripts: Skip exon 4 (Δ exon 4)
Lung Biopsy: Type
Fan et al., *J Pediatr* 1997; 131:565-9

TBB vs VAT vs OLD, immunocompetent children with ILD
Part of prospective study of pediatric ILD, N = 51, 1 m – 18y
21 diagnosis made without biopsy

<table>
<thead>
<tr>
<th></th>
<th>VAT</th>
<th>OLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median</td>
<td>26.5</td>
<td>27</td>
</tr>
<tr>
<td>OR Time, min</td>
<td>35</td>
<td>60</td>
</tr>
<tr>
<td>Chest tube, h</td>
<td>23</td>
<td>63</td>
</tr>
<tr>
<td>Hospitalization, h</td>
<td>36</td>
<td>96</td>
</tr>
</tbody>
</table>

Diagnostic yield higher in older (> 24 m): 12/14 vs 3/13
Biopsy approach: VATS

VATS is feasible, even in young infants

21 subjects for DLD, age 11 d – 15 y
2 converted to mini-thoracotomy
8 / 21 with post-op chest tube (remained 1-5d, median 2)

Adequate specimen in all

Diagnoses:
- Pulmonary Hypertension 1
- Lymphangectasia 1
- BPD 1
- PCH 1
- CPI, DIP, NSIP, CIP 8
- Hypersensitivity Pneumonitis 2
- Bronchiolitis Obliterans 2
- Diffuse alveolar damage 2
- Mycobacterium in immunocompromised 3
When: feasibility on ECMO
Houmes et al. *J Ped Surg*, 2017

Retrospective, 1990 – 2014, 1008 Pediatric ECMO patients
25 underwent OLB, 19 neonatal
14 < 7 days on ECMO, 11 > 7 days
All survived procedure; 5 with blood loss > 15 ml/kg

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Survived</th>
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</thead>
<tbody>
<tr>
<td>ACD</td>
<td>6</td>
</tr>
<tr>
<td>PIG</td>
<td>2</td>
</tr>
<tr>
<td>CPI</td>
<td>1</td>
</tr>
<tr>
<td>MAS</td>
<td>3</td>
</tr>
<tr>
<td>PNA</td>
<td>2</td>
</tr>
<tr>
<td>PH</td>
<td>4</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>2</td>
</tr>
</tbody>
</table>

Support withdrawn 6 0
Steroids given 6 2
No change in treatment 13 6

Overall survival low 2\textsuperscript{nd} underlying condition
Interpretation of Lung Biopsy Results

Diffuse Lung Disease in Young Children
Application of a Novel Classification Scheme
Gail H. Deutsch, Lisa R. Young, Robin R. Deterding, Leland L. Fan, Sharon D. Dell, Judy A. Bean, Alan S. Brody, Lawrence M. Nogee, Bruce C. Trappell, Claire Langston, and the Pathology Cooperative Group; Eric A. Albright, Frederic B. Akin, Peter Baker, Pauline M. Chou, Carlyne M. Cool, Susan C. Coventry, Ernest Cutz, Mary M. Davis, Megan K. Dishop, Csaba Galambos, Kathleen Patterson, William D. Travis, Susan E. Wert, and Frances V. White; on behalf of the chILD Research Co-operative

Diffuse Lung Disease in Biopsied Children 2 to 18 Years of Age
Application of the chILD Classification Scheme
Leland L. Fan, Megan K. Dishop, Csaba Galambos, Frederic B. Akin, Frances V. White, Claire Langston, Deborah R. Liptzin, Miranda E. Kroehler, Gail H. Deutsch, Lisa R. Young, Geoffrey Kurland, James HAgood, Sharon Dell, Bruce C. Trappell, and Robin R. Deterding; for the Children’s Interstitial and Diffuse Lung Disease Research Network (chILDRN)

Diffuse Lung Disease in Infancy:
A Proposed Classification Applied to 259 Diagnostic Biopsies
CLAIRe LANGston AND MEGAN K. DISHOP
Department of Pathology, Baylor College of Medicine and Texas Children’s Hospital, 6621 Fannin Street, Houston, TX 77030, USA
Received November 14, 2008; accepted January 26, 2009; published online March 26, 2009.

Review
Diffuse lung disease of infancy: a pattern-based, algorithmic approach to histological diagnosis
Jane E Armes, William Mifsud, Michael Ashworth

J Clin Pathol 2015; 68:100-110
Algorithm for approach to DLD

Pattern recognition, experienced pathologist

Communication with clinician important

Accept the unknown

Armes et al., *J Clin Pathol* 2015; 68:100-110
Caveats: Different etiologies can look similar

CPI

ABCA3

PAP

SP-B

DIP

SP-C

PAP

ABCA3
Same disorder (ABCA3) can look different

FT-Newborn, RDS, Acinar Dysplasia
Y1515X \ Y1515X

2 y/o, ChILD, Lipoid Pneumonia
R20L \ L960S

6 y/o, ChILD, NSIP
G210V \ ΔF1203

15 y/o, ILD, UIP
Exon 4 splice \ H1255Q
# Special Stains

<table>
<thead>
<tr>
<th>Stain</th>
<th>Carbohydrate</th>
<th>Glycogen</th>
<th>Glycoproteins</th>
<th>Collagen</th>
<th>Elastin</th>
<th>Fibrosis</th>
<th>Pulm Htn</th>
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<tbody>
<tr>
<td>PAS</td>
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<td>Trichrome</td>
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<tr>
<td>Pentachrome</td>
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</table>

Specific antibodies for different cell types

- **Endothelial**: CD31 (PECAM-1)
- **Epithelial**: Cytokeratins
- **Macrophages**: CD68

B or T cell specific

Specific antibodies for antigen of interest (Bombesin)
Immunohistochemical staining

<table>
<thead>
<tr>
<th>Mature SP-B</th>
<th>ProSP-B</th>
<th>ProSP-C</th>
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</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Mature SP-B" /></td>
<td><img src="image2.png" alt="ProSP-B" /></td>
<td><img src="image3.png" alt="ProSP-C" /></td>
</tr>
</tbody>
</table>

SP-B Deficient?

- **P1**: Yes – Homozygous SFTPB Mutation
- **P2**: No – Biallelic ABCA3 Mutations
Immunostaining as adjunct to diagnosis: NEHI and Bombesin
Young et al., Chest 2011; 139:1060-1071

Increased NEBs in BPD
Pulmonary Interstitial Glycogenosis

A spectrum of PIG is seen in a variety of settings

- Lung growth abnormality (~50% chILD review cases)
- Pulmonary hypertension
- Other (meconium, congenital lobar emphysema, CPAMs.....)

Growth + patchy PIG

PIG is a finding, not a diagnosis!

Slide courtesy of Gail Deutsch, Seattle Children’s
Lung Growth Abnormalities

Control

Pulmonic Stenosis

A

B

C

D

Trisomy 21

27 weeks GA

Deutsch et al., AJRCCM, 2007
Lung Biopsy: Pulmonary Vascular Disease

- Pulmonary artery medial hypertrophy associated with Congenital Heart Disease
- Lymphangectasia
- Hemorrhage and Pulmonary capillaritis

Dishop, MK. Pediatr Allergy Immunol Pulm 2010; 23:69-85
Lymphoid hyperplasia
NSIP
Lymphocytic IP
Interstitial fibrosis
Pulmonary arterial disease
Pleural disease

JIA
Cellular and fibrosing NSIP
Lymphocytic infiltrate

Dishop, MK. *Pediatr Allergy Immunol Pulm* 2010; 23:69-85
Summary \ Take Home Points

Lung biopsy remains an important diagnostic tool.

Published protocols exist for optimal handling and processing.

- Make maximal use of precious material (EM, frozen)

Good communication between clinicians, imagers, surgeons and pathologist is essential for optimal results.

Biopsy is feasible and can be useful in children on ECMO.

Don’t be afraid to share: biopsy is usually done because it is a difficult diagnosis.
References


Classification of Childhood ILD/DLD

Developmental Disorders
- Congenital Alveolar Dysplasia
- Alveolar Capillary Dysplasia

Growth Disorders
- Pulmonary Hypoplasia
- BPD
- Chromosomal Abnormalities
- Congenital Heart Disease

Surfactant Dysfunction Disorders
- Acinar Dysplasia
- NKX2-1
- ABCA3
- Unidentified

Masqueraders
- Lymphatic Abnormalities
- Pulmonary Hypertension
- Congestive Heart failure
- Veno-occlusive disease

Immunocompromised
- Opportunistic Infection
- Therapy related
- Transplant, rejection

Normal Host
- Infection
- Environmental
- Aspiration
- Eosinophilic Pneumonia
- IPH

Systemic Disease
- Immune-mediated
- Storage
- Sarcoidosis
- Langerhans Histiocytosis

Unidentified
- End stage
- Inadequate sample
- Non-diagnostic

Disorders unique to infancy of unknown etiology
- PIG
- NEHI
- PI

Lymphatic Abnormalities
- Pulmonary Hypertension
- Congestive Heart failure
- Veno-occlusive disease

Opportunistic Infection
- Therapy related
- Transplant, rejection

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- PI

NEHI
- PI
- PIG

Disorders unique to infancy of unknown etiology
- PIG
- NEHI
- PI
Diffuse Developmental Disorders

Acinar Dysplasia

- Developmental arrest in the pseudoglandular stage
- Nearly absent acinar development
- Small lungs with thickened interlobular septa

Slide courtesy of Gail Deutsch, Seattle Children’s
Congenital Alveolar Dysplasia

- Developmental arrest in the canalicular/early saccular stage
- Thickened septa; reduced capillary density
- Normal to increased lung weight

Slide courtesy of Gail Deutsch, Seattle Children’s