Lung Biopsy in Children
Patterns, Pearls, and Pitfalls
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Learning Objectives

- List the differential diagnosis of a “normal” lung biopsy.
- Describe the clinical associations with a pattern of alveolar simplification.
- Recognize the histologic patterns associated with the genetic disorders of surfactant metabolism.
- Describe the clinical associations with patterns of pulmonary lymphoid hyperplasia.
- Correlate clinical and radiologic information with histologic patterns on lung biopsy to develop clinicopathologic differential diagnosis.

Pediatric Lung Biopsy: Indications

- Nodules, esp. immunosuppressed and oncology pts.
- Exacerbation or disproportionate severity in chronic neonatal lung disease
- Factors causing pulmonary hypertension – cardiac vs. lung parenchymal vs. intrapulmonary vasculature
- Obstructive airway disease – t/o BO
- Chronic tachypnea – t/o NEHI vs. other
- Cause of ILD – t/o genetic surfactant disorder
  - Inflammation vs. fibrosis
- Common goal: Provide direction for further diagnostic evaluation and therapy.

Questions prior to lung biopsy

- Site of biopsy
  - Affected lobe; region of involvement
  - If diffuse, any lobe – except not the tip of the right middle lobe or lingula.
- Size of biopsy
  - 2-3 cm wide and at least 1 cm deep
  - Sampling of muscular pulmonary arteries and terminal bronchioles

Faculty Disclosure

- Megan K. Dishop: No financial conflicts of interest
**Microscopic examination**

- Anatomic compartments of the lung
  - 1. Alveolar spaces
  - 2. Interstitium
  - 3. Airways (bronchi and bronchioles)
  - 4. Vasculature (arteries, veins, lymphatics)
  - 5. Pleura and interlobular septa
- Use of microscopic description in reporting
- Integration of clinical and radiologic information into final diagnosis and comment

**Patterns in Pediatric Lung Biopsy**

- Normal or near-normal
- Alveolar-filling
- Interstitial cellularity
- Interstitial fibrosis
- Lymphoid proliferation
- Chronic bronchiolitis and bronchiolectasis
- Vasculopathy and vasculitis
- One or more patterns...

**CATEGORY**

**SPECIFIC DISEASE ENTITIES**

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>SPECIFIC DISEASE ENTITIES</th>
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<tr>
<td>Diffuse developmental disorders</td>
<td>Acinar dysgenesis, congenital alveolar dysplasia, alveolar capillary dysplasia with malalignment of pulmonary veins</td>
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<td>Growth abnormalities</td>
<td>Pulmonary hypoplasia, chronic neonatal lung disease, related to chromosomal disorders and congenital heart disease</td>
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<td>Specific conditions of undefined etiology, likely reactive</td>
<td>Neuroendocrine hyperplasia of infancy, pulmonary interstitial glycogenosis/fibroblast cell interstitial pneumonitis</td>
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<td>Surfactant dysfunction disorders and related conditions</td>
<td>SP-A, SP-C, ARCAT, tannin protein intolerance</td>
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<td>Disorders of the normal host</td>
<td>Infectious and post-infectious processes, related to environmental agents, aspiration, eosinophilic pneumonia</td>
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<td>Disorders related to systemic disease processes</td>
<td>Immune-mediated disorders, metabolic disease, Langerhans cell histiocytosis</td>
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<tr>
<td>Disorders of the immunocompromised host</td>
<td>Opportunistic infections, related to therapeutic intervention, transplantation and rejection</td>
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<td>Disorders masquerading as interstitial disease</td>
<td>Arterial, venous, lymphatic disorders; congestive changes related to cardiac dysfunction</td>
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<tr>
<td>Unclassified</td>
<td>End-stage lung disease, non-diagnostic, inadequate</td>
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**Normal or near-normal lung biopsies in children**
Normal or near-normal pattern

- Abnormal alveolarization ("alveolar simplification")
  - Look for deficiency of alveolar septation
  - Correlate with clinical hx and chest CT
  - Associations:
    - Prematurity
    - Hypoplasia
    - CHD
    - Chromosomal
    - Neonatal injury
    - TTF1/NKX2.1

Case: Persistent tachypnea

- 3 month old term infant with tachypnea noticed 3 weeks prior to presentation.
  - RSV infection at 1 month of age.
  - Normal echocardiogram.
  - CXR hyperinflation.
  - CT patchy ground glass opacities.

Neuroendocrine cell hyperplasia of infancy (NEHI)

- Persistent tachypnea of infancy
- Oxygen requirement for months-years
- Diagnosis:
  - Increased airway neuroendocrine cells (>10%+)
    - Normal 5-6%
    - Bombesin stain vs. chromogranin, synaptophysin
  - Large neuroepithelial bodies
  - Exclusion of other histologic patterns
    - Lack of significant airway fibrosis, interstitial disease or vasculopathy
  - Typical clinical and imaging features
- Etiology unknown
  - Developmental vs. post-inflammatory
Obliterative bronchiolitis

- Bronchiolitis obliterans syndrome (BOS)
- PFTs – obstructive
- CT – mosaic perfusion, regional air-trapping
- Differential diagnosis:
  - Post-viral sequela - adenovirus
  - Chronic aspiration
  - Stevens-Johnson syndrome
  - Chronic airway rejection – lung transplant
  - Chronic GVHD – bone marrow transplant

- Histology:
  - Airway fibrosis (constriction or obliteration of lumen)
  - ‘Unpaired’ pulmonary arteries
  - Mucus stasis
  - Distension of airspaces/alveolar ducts
  - Periairway foamy macrophages, cholesterol clefts

- Sampling error
Aspiration injury

- Difficult diagnosis – commonly, non-specific findings
  - BAL lipid-laden macrophages
  - Airway-associated lymphoid hyperplasia
  - Organizing pneumonia
  - Endogenous lipid pneumonia
- Foamy macrophages/cholesterol
- Other clues:
  - Granulomas and food particles
  - Exogenous lipid pneumonia – mineral oil
Case. DIP/PAP in infant

- 6 week old girl with hypoxia and respiratory insufficiency
  - Term gestation, discharged on dol 5
  - CXR diffuse infiltrates
  - CT ground glass opacities in “crazy paving” pattern, septal thickening
  - Lung biopsy performed

Alveolar filling and Interstitial cellularity in children
Surfactant Dysfunction Disorders

**ABCA3**
- Expressed on surface of lamellar bodies
- Lipid transport
- Abnormal processing of lamellar bodies

**Surfactant Dysfunction Disorders**

- **Etiology**
  - Genetic mutations in surfactant proteins
    - SP-B (SFTPB, chr 2p12-p11.2, AR)
    - SP-C (SFTPC, chr 8q21, AD)
  - Genetic mutations in ATP binding cassette transporter
    - ABCA3 (chr 16p13.3, AR)
  - *Now*, the most common cause of surfactant dysfunction disorders
  - Surfactant dysregulation
    - TTF1/NKX2-1 mutations/deletions
    - GM-CSF receptor mutations
      - CSF2RA (X-linked)
      - CSF2RB (chr 22)

**Surfactant Dysfunction Disorders: Histologic Spectrum**

**Histologic Patterns in Infancy**
- Pulmonary alveolar proteinosis pattern (SPB, ABCA3)
- Desquamative interstitial pneumonia pattern
- Chronic pneumonitis of infancy pattern (SPC)

**Histologic patterns in Older children and Adults**
- Nonspecific interstitial pneumonia (ABCA3, SPC)
- Pulmonary fibrosis (SPC)

**Spectrum of patterns**: PAP, DIP, CPI, NSIP, IPF
Chronic Pneumonitis of Infancy
A Unique Form of Interstitial Lung Disease Occurring in Early Childhood

Anna-Louise A. Katzstein, M.D., Lawrence P. Gordin, M.D.,
Michael Oliphant, M.D., and Philip T. Swenson, M.D.


ABCA3 disease

Older children, adolescents
• 10-25 years old.

ABCA3 Mutations associated with pediatric ILD

6 year old girl with possible Hypersensitivity pneumonitis
Nonspecific interstitial Pneumonia (NSIP) pattern

7 month old Known SPC mutation

6 year old girl with possible Hypersensitivity pneumonitis
Nonspecific interstitial Pneumonia (NSIP) pattern
**ABCA3 mutations – endogenous lipoid pneumonia pattern**

**TTF1/NKX2.1 deficiency**
- "Brain-lung-thyroid syndrome"
- Clinical clues to diagnosis
  - Hypothyroidism
  - Neurologic symptoms
- Early transcription factor in lung development
- Regulates production of surfactant proteins
- Variable histopathology
  - "Normal"
  - Alveolar growth abnormality
  - Pulmonary alveolar proteinosis/chronic pneumonitis of infancy

**Surfactant Dysfunction Disorders: Ultrastructural Features**
- SP-B mutations – Abnormal lamellar bodies; Multivesicular and multilamellated bodies.
- ABCA3 mutation – Abnormal dense bodies ("fried egg")
- SP-C mutation – Normal or non-specific lamellar body structure.
- TTF1/NKX2-1 mutations – Normal or non-specific lamellar body structure.
Normal

SP-B

Abca3 mutations

Acquired pulmonary alveolar proteinosis (AML on therapy)

Pitfall: organizing hyaline membranes or fibrin aggregates

Pitfall: Epithelial cell necrosis from overwhelming respiratory viral infection (RSV in SCID)
Case. Pulmonary fibrosis in adolescent

- 14 year old girl presents for lung transplantation
  - Respiratory insufficiency beginning in infancy
  - Biopsy at 2 years of age: CPI
  - CT shows honeycomb fibrosis with septal thickening and cystic changes.
  - Family history:
    - Father recently diagnosed with chronic lung disease in 40’s; non-smoker
    - Paternal grandfather died of idiopathic pulmonary fibrosis

Interstitial fibrosis in children
Pulmonary fibrosis in children

- Bronchopulmonary dysplasia
  - Historic; improves over time
- Organizing diffuse alveolar damage
- Chemoradiation
  - Subpleural and paraseptal (XRT)
-ILD with progressive changes
  - Genetic disorders of surfactant metabolism (ABCA3, SFTPC)
  - Chronic hypersensitivity pneumonitis
  - Collagen vascular disease (scleroderma, DM, MCTD, other)
- Other rare disease: DNA repair defects

Case. Pulmonary nodules

- 4 year old girl presents with mild cervical lymphadenopathy, splenomegaly, and diarrhea
- GI biopsies show non-specific lymphonodular hyperplasia
- Cough and hypoxia
- CT shows multiple bilateral nodules, ground glass opacities, and mild bronchiectasis
- Lung biopsy
Pulmonary lymphoid hyperplasia

- Primary immunodeficiency
  - CVID, XLP
- Acquired immunodeficiency (HIV)
- Immune dysregulation
  - Autoimmune lymphoproliferative syndrome (ALPS)
    - Mutations in FAS gene
    - Double-negative (CD4-CD8-) T cells
  - Autoimmune polyendocrinopathy syndrome (APS)
    - Mutations in autoimmune regulator (AIRE) gene
    - Autoantibodies to various organs, including lung
  - Hemophagocytic lymphohistiocytosis
- Autoimmune disease (collagen vascular disease)
- Rule out lymphoma/leukemia
Common variable immunodeficiency
Patterns:
• Follicular bronchiolitis
• Lymphoid interstitial pneumonia (LIP)
• Granulomatous-lymphocytic interstitial lung disease (GLILD)

Collagen vascular disease
• General histologic patterns
  - Lymphoid hyperplasia
  - Lymphoid interstitial pneumonia (LIP)
  - Non-specific interstitial pneumonia (NSIP)
    - Differential Dx: CVD, HP, ABCA3/SP-C mutations
    - Increased interstitial plasma cells
    - Lymphocytic/constrictive bronchiolitis
    - Vasculopathy
    - Vasculitis
    - Pleuritis/pleural fibrosis

Pulmonary hemosiderosis in children

Pulmonary hemosiderosis
• Accumulation of hemosiderin-laden macrophages
  - When this is the predominant pattern, consider the following differential diagnosis:
    • Vasculitis (arteritis, capillaritis)
    • Vasculopathy (pulmonary arteriopathy, chronic congestive vasculopathy)
    • Other interstitial lung disease
    • Resolving hemorrhage related to acute lung injury
    • Idiopathic pulmonary hemosiderosis
Pulmonary hemosiderosis

- Accumulation of hemosiderin-laden macrophages
  - Features that suggest a vasculitis or acute capillaritis:
    - Repeated episodes of hemorrhage
    - Anemia (acute drop in hemoglobin/hematocrit)
    - Fluffy bilateral infiltrates or consolidation on chest x-ray
    - Positive serology: ANA, PR3 (c-ANCA), MPO (p-ANCA)
      - Helpful if present. Negative serology does not exclude diagnosis.

Pulmonary hemorrhage and hemosiderosis

- "Soft signs" of vasculitis
  - Fibrin – low power clue to areas of capillaritis
  - Organizing pneumonia – implies alveolar wall damage and repair
  - Lymphoid hyperplasia – implies immunologic activation
  - Look for increased interstitial or alveolar neutrophils, greater than background circulating neutrophils
    - Karyorrhexis (leukocytoclasis) or necrosis is helpful
Clinical correlation is important
- Exclude renal hemorrhage (ex. Crescentic glomerulonephritis), other systemic vasculitis
- Serologic testing
  - P-ANCA (MPO) – most often with microscopic polyangiitis, isolated pulmonary capillaritis
  - C-ANCA (PR3) – most often with granulomatosis with polyangiitis (Wegener granulomatosis)
  - May be negative, even during active vasculitis.
  - May become positive later in disease course.
- Pre-treatment with steroids
  - May diminish neutrophils in biopsy, and therefore lead to descriptive or “suggestive of” diagnosis
  - Sampling error

Chronic congestive vasculopathy
- Hemosiderosis
- Congestion
- Alveolar hemorrhage
- Venous thickening or arteriolization
- Lymphatic muscularization

Idiopathic Pulmonary Hemosiderosis
Hemosiderosis only
Clinical diagnosis of exclusion
No vasculopathy or vasculitis in biopsy
Cannot rule out inactive or treated vasculitis...

Summary: Pattern-based Differential Diagnosis
- Normal or near-normal
- Alveolar-filling
- Interstitial cellularity
- Interstitial fibrosis
- Lymphoid proliferation
- Chronic bronchiolitis and bronchiolectasis
- Vasculopathy
- One or more patterns...
Abnormal alveolarization
- Look for deficiency of alveolar septation
- Correlate with clinical history and chest CT

Neuroendocrine cell hyperplasia of infancy
- Bombesin stain

Obliterative bronchiolitis
- Movat pentachrome or elastic trichrome

Abnormal vascular flow
- Edema fluid washed out
- Consider pulmonary overcirculation, capillary leak, AVM or telangiectasia, shunt physiology

Pulmonary alveolar proteinosis
- Genetic surfactant disorders, GM-CSF antibody, macrophage dysfunction (immunologic impairment)

Macrophages (desquamative interstitial pneumonia)
- Genetic surfactant disorders, drug reaction, inhalational injury
- Hemosiderin: if abundant, consider chronic congestive vasculopathy, vasculitis
- Foamy macrophages: aspiration, surfactant disorder, resolving pneumonia, airway obstruction, storage disorders

Neutrophils
- Bacterial pneumonia, capillaritis

Lymphocytes/histiocytes
- Hypersensitivity pneumonitis, sarcoidosis, immunologic disease
- Organizing pneumonia
- Causes of alveolar wall injury
  - Infection, aspiration, HP, ILD, idiopathic (cryptogenic)

Macrophages
- Storage disease, histiocytosis

Neutrophils
- Infection, capillaritis, SLE

Eosinophils
- Eosinophilic pneumonia, Churg-Strauss syndrome, parasitic infection, foreign body response

Lymphocytes
- Viral or aspiration pneumonitis, hypersensitivity pneumonitis, surfactant disorders

Plasma cells
- Autoimmune/collagen vascular; some viral

Interstitial fibrosis pattern
- Fibrosis +/- cellularity
  - Genetic surfactant disorders
  - Collagen vascular disease
  - Hypersensitivity pneumonitis
  - Resolved org DAD
  - Chemotherapy/radiation injury
  - DNA repair defects
Lymphoid proliferation patterns

- Follicular bronchiolitis
  - Congenital or acquired immunodeficiency
  - Autoimmune disease
  - EBV infection
  - Idiopathic
- Lymphoid interstitial pneumonia (LIP)
  - Congenital or acquired immunodeficiency (esp. HIV)
  - Autoimmune (esp. Sjogren syndrome)

Chronic bronchiolitis or bronchiolectasis patterns

- Lymphocytic bronchiolitis
  - Respiratory viral infection, PCD, immunodeficiency
  - GVHD (BMT), ACR (lung tx)
- Constrictive/obliterative bronchiolitis
  - Consider pentachrome or elastic trichrome
  - s/p necrotizing bronchiolitis: viral, MFS
  - GVHD (BMT), ACR (lung tx)
- Chronic bronchiolitis with acute inflammation
  - Cystic fibrosis
  - Secondary infection in chronic airway disease

Pulmonary vasculopathy patterns

- PA medial hypertrophy
  - Assoc. with ILD or alveolar simplification
  - Assoc. with congenital heart disease
  - ACD, pulmonary venous disease
- PV arterialization (chronic congestive vasculopathy)
  - Impaired pulmonary venous outflow or elevated left cardiac pressures
  - Correlate with echo and cath. R/O TAPVC, PVS, CMP
- PVOD
  - Use elastic trichrome or pentachrome to highlight obliterated veins

Questions and Discussion