

# **Diagnostic Electron Microscopy in the Evaluation of Thoracic Tumors: Continuing Indications.**

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## **Introduction**

The compartments of the thorax (lungs, serosal membranes, mediastinum and chest wall) give rise to myriad neoplasms, benign and malignant, incorporating all facets of histogenesis. These challenge the pathologist to make a correct diagnosis, as the tumors at these sites retain significant potential to mimic one another, in terms of gross distribution as well as histomorphology. Diagnostic accuracy is critical, as prognostication and therapeutic decisions vary considerably amongst the various thoracic neoplasms. The pathologist's armamentarium has evolved considerably over the past several decades, and correct diagnosis is often made possible through techniques adjunctive to histologic examination, including histo- and immunohistochemistry, as well as molecular and cytogenetic studies. Examination of tumor ultrastructure also provides useful information for accurate diagnosis in complex or unusual cases, or in instances when ancillary diagnostic studies yield equivocal results. While not exhaustive, several diagnostic indications will be reviewed in this presentation.

## **Specific Indications**

### **1. Malignant (diffuse) Pleural Mesothelioma**

- A. Primary differential diagnostic consideration: "Pseudomesotheliomatous" pulmonary adenocarcinoma, metastatic clinically occult carcinoma (e.g. renal cell, breasts).
- B. Light microscopy pitfalls: mucin-positive (D-PAS, mucicarmine) mesotheliomas, aberrant coexpression of adenocarcinoma immunohistochemical markers by mesothelioma, or converse
- C. Diagnostic ultrastructural features: High aspect ratio surface microvilli, perinuclear tonofilaments, hyaluronic acid crystals
- D. Exclusionary features: Mucous granules, lamellar bodies, dense core granules
- E. Limitations: Sarcomatoid/Desmoplastic mesotheliomas- may demonstrate ultrastructural features suggestive of fibro-myofibroblastic differentiation, rarely show intercellular junctions or surface microvilli

## **2. Tumors of Vascular Differentiation (Angiosarcoma, Epithelioid Hemangioendothelioma)**

- A. Primary differential diagnostic consideration: Metastatic adenocarcinoma, malignant pleural mesothelioma, monophasic synovial sarcoma
- B. Light microscopy pitfalls: Confusion of intracytoplasmic lumina with mucin (EHE), aberrant coexpression of cytokeratins, focal (angiosarcoma)
- C. Diagnostic ultrastructural features: well-developed basal lamina, pinocytotic vesicles, numerous intermediate filaments, Weibel-Palade bodies

## **3. Small Round Cell Tumors/Neuroendocrine Tumors of Adulthood (Primitive Neuroectodermal Tumor/Askin Tumor)**

- A. Primary differential diagnostic consideration: Lymphoma, small cell carcinoma, rhabdomyosarcoma
- B. Light microscopy pitfall: CD99 and cytokeratin expression
- C. Diagnostic ultrastructural features: dense core granules, neurotubules, neuritic processes-common features of neuroendocrine differentiation

## **4. Histiocytic Neoplasms (Pulmonary Langerhans Histiocytosis)**

- A. Primary differential diagnostic consideration: Metastatic disease, non-specific interstitial scarring in cases of “burnt out” disease, histiocytic and dendritic cell neoplasms, Hodgkins lymphoma
- B. Light microscopy pitfall: non-specific S-100 staining, variable persistence of Langerhans histiocytes in chronic lesions
- C. Diagnostic Ultrastructural features: Birbeck granules

## **5. Miscellaneous tumors ( Synovial Sarcoma, Alveolar Soft Part Sarcoma, Glomus Tumors)**

### **A. Synovial Sarcoma**

- 1. Primary differential diagnostic consideration: Primary or metastatic carcinoma, mesothelioma, other sarcoma
- 2. Pitfall: Protean histologic appearance, mono- and biphasic forms, coexpression of cytokeratins and calretinin
- 3. Diagnostic ultrastructural features: biphasic forms show distinct epithelial and spindled populations, the former feature gland-like structures with microvilli, paranuclear aggregates of

intermediate filaments, rare tonofilaments. Spindle cells have ultrastructural features of fibroblasts, may have evidence of rudimentary epithelial differentiation, poorly formed junctions, desmosome-like structures, microvilli

#### B. Alveolar Soft Part Sarcoma

1. Primary differential diagnostic consideration: Tumor predominantly of young adulthood involving chest wall, may be confused with other sarcomas, metastatic carcinoma, germ cell neoplasm

2. Diagnostic ultrastructural features: numerous mitochondria, prominent SER, well-developed Golgi apparatus and rhomboid, rod-shaped or spicular crystalline material

#### C. Glomus Tumors:

1. Primary Differential Diagnosis: Rarely arising in lung, airway, mediastinum, diagnostic considerations include low grade neuroendocrine neoplasms (carcinoid, paraganglioma), granular cell tumor, hemangiopericytoma

2. Diagnostic ultrastructural features: Closely related to smooth muscle cells: Thick basal lamina, bundles of thin, actin-like nuclei, myofilaments. Oncocytic forms contain numerous mitochondria

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**Society for Ultrastructural Pathology Companion Meeting - February 27, 2011**

**Symposium on Interface of Pulmonary and Ultrastructural Pathology:  
Metals, Minerals and Morphology**

**Pediatric Pulmonary Neoplasia: Current Perspectives**

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**Pediatric Neoplasia: Background**

**Primary Lung Tumors in Neonates, Infants, Children and Adolescents**

- Rare Tumors
  - 0.2% of All Childhood Tumors
  - Malignant Tumors (65-76%) More Common than Benign Tumors (24-35%)
  - Mortality for Benign Tumors 8% and Malignant Tumors 30-50%
- Metastatic Tumors 5-times More Common
- Benign Non-Neoplastic Tumors 60-times More Common Than Primary Tumors
  - Bronchogenic Cysts and Bronchial Atresia with Mucocele
  - Pulmonary Sequestrations (intralobar, extralobar)
  - Congenital Cystic Adenomatoid Malformations.
- Pulmonary Infections, Inflammatory or Reactive Processes May Mimic Solid Tumors

**Symptoms Associated with Pediatric Lung Tumors**

	Benign (n=86)	Malignant (n=255)
• None	28%	6%
• Fever	16%	18%
• Cough	14%	35%
• Pneumonitis	10%	23%
• Chest Pain	8%	7%
• URT Infection	7%	2%



- Leiomyosarcoma (4%: most associated with EBV)
- Angiosarcoma and Kaposi Sarcoma (HHV8 associated)
- Mesothelioma

### **Metastatic Tumors Involving Lung**

- Secondary Involvement of Lung
  - Leukemic or Lymphomatous Infiltrates
    - Lymphoproliferative Disorders, Monomorphic
    - Hodgkin and Non-Hodgkin Lymphomas
    - Leukemias: ALL, AML, CML
- Solid Tumors
  - Wilms Tumor, Osteosarcoma, Ewing Family of Tumors
  - Rhabdomyosarcoma, Germ Cell Tumors
  - Neuroblastoma, Hepatoblastoma, Hepatocellular Carcinoma
  - Rhabdoid Tumor, Clear Cell Sarcoma of Kidney
  - Cellular Mesoblastic Nephroma
  - Desmoplastic Small Round Cell Tumor
  - Undifferentiated Sarcoma, Synovial Sarcoma, Angiosarcoma
  - Clear Cell Sarcoma of Soft Tissue, Fibrosarcoma, Liposarcoma
  - Myofibrosarcoma, Pleomorphic Undifferentiated Sarcoma (MFH)
  - Alveolar Soft Part Sarcoma
  - Nasopharyngeal Carcinoma, Adenoid Cystic Carcinoma
  - Colon Adenocarcinoma, Clear Cell Carcinoma, Squamous Cell Carcinoma
  - Juvenile Secretory Carcinoma of the Breast

### **Selected Primary Pediatric Lung Tumors**

#### **Fetal Lung Interstitial Tumor (FLIT)**

- Newly Recognized Lung Tumor of Infancy (1st Case Diagnosed as Atypical PPB)
- Currently Reported: 10 Infants (7 Females, 3 Males) with Tumor-Like Lung Masses
- Detected in Perinatal Period and up to 3 Months of Age (median 1 day-old)
- Often Detected by Prenatal Ultrasound
- Symptoms
  - Variable Respiratory Distress Shortly After Birth (mild to moderate to severe)
  - Progressive Feeding Difficulties
  - Low Grade Fever
  - Diminished Breath Sounds
  - Airway Obstruction with "Inspissated Mucin"
- Radiologic Imaging
  - Well Circumscribed, Solid or Mixed Solid/Cystic Lobar-Based Mass
  - Solid Masses in 8/10 Cases and Partially Cystic Masses in 2/10 Cases
- Pathologic Features
  - Gross Appearance:
    - Tumor Size: 2 to 7 cm
    - Well Circumscribed, Tan-Pink to Dark Red-Brown
    - Solid to Spongy Mass

- Border Between Mass and Normal Lung Demarcated by Complete to Incomplete Fibrous Interface
- Histopathologic Appearance:
  - Comprised of Immature Mesenchyme Associated with Irregular Airspace-Like Structures Resembling Abnormal Incompletely Developed Lung
  - Gestationally Inappropriate, Immature Airspaces Formed by Variably Widened Septa with Similarly Immature Interstitial Cells
  - Interstitial Compartment Comprised of Monotonous Immature Round to Oval Mesenchymal Cells.
  - Interstitial Cells Formed Uniform Monolayer of Polygonal Cells Without A Cambium Layer Beneath the Non-Ciliated, Low Cuboidal to Flattened Epithelium
  - Interstitial Cells Possessed Clear to Pale Eosinophilic Cytoplasm with PAS Granular Positivity that Digested with Diastase (glycogen) and Nuclei with Dispersed Chromatin and Inconspicuous Nucleoli
  - Membranous Bronchiolar and Small Airway Structures Modest in Number and Accompanied by Smooth Muscle.
  - Isolated Foci of Cartilage Similar to That in Small Bronchioles in 2 Cases
  - Less Frequent Pattern (2/10 Cases) of Immature Fibroblastic to Myofibroblastic Cells Replacing Polygonal Interstitial Cells with Focal Chronic Inflammatory Cells Including Plasma Cells
  - Normally Developed Lung Present in Immediate Vicinity to the Mass
  - Fibrous Border or Abrupt Interface Demarcated Normal Saccular Stage Lung From Masses Immature Airspace-Like Structure with Widened Cellular Septa
  - Resembles Fetal Lung at 20 to 24 Weeks Gestation Canalicular Stage
- Immunocytochemistry:
  - Positive for Strong Diffuse Vimentin, Focal SMA and Focal Desmin For Interstitial Cells
  - Negative for Myogenin
  - Epithelial Lining of Airspaces: Positive for Cytokeratin, EMA and TTF-1
  - Mib1 (Ki-67): 15-25% of Interstitial and Epithelial Cells Positive
- Electron Microscopy;
  - Plump Alveolar Epithelial Lining Cells with Abundant Cytoplasmic Glycogen and Some with Lamellar Bodies Consistent with Type II Alveolar Cell Differentiation
  - Interstitial Cells with Abundant Cytoplasmic Glycogen and Rare Fibronexus Structures Consistent with Myofibroblastic Differentiation
  - Smooth Muscle Cells Present Adjacent to Epithelial Basement Membranes
- Cytogenetics:
  - Normal Karyotype in 2 Cases (Not Done in 8 Cases)
  - DICER1 Sequencing Negative in 1 Case

- Lobectomy or Wedge Resections in Each Case With Complete Excision in 8/10 Cases
- *Ex Utero* Intrapartum Surgical Resection at 37 Weeks Gestation in 1 Case with MRI Indicating Fetal Ascites Due to Inferior Vena Caval Obstruction
- Only 1 Case with Vincristine-Based Chemotherapy Initiated Although Diagnosis of FLIT was Made at Time of Resection and Not PPB.
- No Recurrences and No Metastatic Disease in All Cases on Follow-Up after 15-182 months
- Differential Diagnosis: Bronchogenic Cyst, Congenital Cystic Adenomatoid or Pulmonary Airway Malformation (CCAM-CPAM), Cystic Pleuropulmonary Blastoma (PPB Type I), Pulmonary Interstitial Glycogenosis (PIG), Congenital Peribronchial Myofibroblastic Tumor (CPMT)
  - FLIT
    - Gross: Solid to Spongy Lobar Based Mass
    - Microscopic:
      - Circumscribed Lesion with Partial Fibrous Interface with Adjacent Compressed Normal Lung
      - Small Airspace-Like Structures Lined by Single Layer of Flattened to Cuboidal Epithelium
      - Septal Interstitial Expansion by Uniform Polygonal Cells with Clear Cytoplasm (PAS Positive, Diastase-Digestion)
      - Network of Delicate Capillaries
    - Lung Involvement: Limited to Single Lobe
  - Cystic Pleuropulmonary Blastoma (PPB Type I)
    - Gross: Collapsible, Delicate Multicystic Structure Located at Periphery of Lobe
    - Microscopic:
      - Transition from Normal Lung to Variable-Sized Cysts with Continuous or Discontinuous Population of Primitive Small Cells with or without Rhabdomyoblastic Differentiation Beneath Lining Epithelium (Cambium Layer)
      - Small Nodules of Primitive Cartilage (not all cases), Fibrous Interstitium with Prominent Vessels, Focal Necrosis and/or Hemorrhage
    - Lung Involvement: Multifocal in 40% of Cases
  - Congenital Cystic Adenomatoid Malformation
    - Gross: Dominant Lobar-Based Cyst or Variable-Sized Cysts Within Lung Parenchyma (Types I and 2)
    - Microscopic:
      - Dominant Cyst Lined by Ciliated Respiratory Epithelium With Adjacent Compressed Lung (type 1) or
      - Multiple Variable-Sized Cysts Lined by Respiratory Epithelium (type 2)
      - Background of Normal Lung Parenchyma
      - Immature But Differentiated Rhabdomyomatous Cells in Interstitium (type 2)
      - Microscopic Terminal Bronchiole-like Structures with Solid Gross Appearance (type 3)
    - Lung Involvement: Rarely Multilobar in  $\leq 1\%$  of Cases

## **Pleuropulmonary Blastoma (PPB)**

- Embryonal Malignant Tumor Derived from Mesenchyme of Lung and Pleura
- Rare Tumor with 20 to 25 Cases Per Year in USA
- First Described in 1988 as Distinct Entity
- Predominantly in Neonates, Infants and Young Children (single documented adult case reported)
- Rarely Reported After 12 years of Age
- Detection May Occur During Routine Ultrasound Prenatally
- Important to Distinguish from Adult Pulmonary Blastoma
  - Adult Pulmonary Blastoma: Biphasic Tumor with Both Malignant Mesenchymal and Epithelial (glandular) Components (Carcinosarcoma)
  - PPB: Only Malignant Mesenchymal Component and No Malignant Epithelial Component
- Equal Gender Ratio
- Laterality: Right Lung 54%; Left Lung 37%; Bilateral 9%)
- PPB Types (I, II, III and IR)
  - Type I PPB (Purely Cystic Tumors): 27% of All PPBs
    - Occur in Youngest Affected Patients (median age 10 months, range newborn to 32 months)
    - Multilocular Cyst on Radiologic and Gross Examination
    - Respiratory Distress Due to Air-Filled Cysts or Pneumothorax
    - Usually Incidental Lung Cysts on Chest X-Ray - Often Confused with Congenital Cystic/Pulmonary Adenomatoid Malformation (CCAM/CPAM)
    - Pathology of Type I PPB
      - Single or Multiloculated Cysts with Thin Fibrous Septa
      - Cysts Lined by Ciliated Columnar Epithelium
      - Subepithelial Small "Buds"/Aggregates of Primitive Mesenchymal Cells and/or Nodules of Immature Cartilage
      - Subepithelial Cambium Layer- Continuous or Discontinuous Condensed Zone
        - Small Round to Spindled Immature/Primitive Mesenchymal Cells Forming Cambium Layer
        - Botryoid Appearance with Intermixed Polygonal and Strap Cell Rhabdomyoblasts
        - Myogenin, Desmin, MyoD1, MSA: Positive
        - Ultrastructure: Myofilaments, Rudimentary Z-bands
    - Differential Diagnosis
      - CCAM Types I, II and III
      - CPAM Type 4
      - FLIT (Fetal Lung Interstitial Tumor)
  - Type II PPB (Solid and Cystic Tumors): 35% of All PPBs
    - Median Age 36 Months (range 15-64 months, single documented 36 year-old)
    - Dyspnea, Fever, Cough, Chest or Abdominal Pain, Pneumonia, Malaise, Anorexia
    - Pleural Effusion, Pneumothorax
    - Pathology: Cystic and Solid Tumors
      - Cystic Component of Type II PPB

- Remnants of Cysts with Thin Fibrous Septa and Lined by Ciliated Columnar Epithelial Cells with Subepithelial Malignant Mesenchymal Cells
  - Predominantly Cystic PPBs with Plaque-Like Areas with Overgrowth of Rhabdomyoblasts, Spindle Cell Sarcoma or Blastematos Elements
- Solid Tumor: Mixed Sarcomatous and Blastematos Features
  - Cellular Islands of Small Primitive Blastematos Cells (oval nuclei, granular chromatin, inconspicuous nucleoli, minimal cytoplasm, numerous mitotic figures)
  - Stroma Blends with Spindle Cells Organized into Vague Fascicular Pattern of Fibrosarcoma or Pleomorphic Undifferentiated Sarcoma (MFH)
  - Stroma Resembling that Surrounding Blastema in Wilms Tumors
  - Foci of Skeletal Muscle and Chondroid Differentiation (resembles rhabdomyosarcoma or chondrosarcoma or fetal/immature cartilage)
  - Foci of Anaplasia with Giant Bizarre Pleomorphic Tumor Cells in Many Type II and III PPBs
  - "Cystic" Necrosis in Solid PPB Areas -Friable Empyema-like Tissue
  - Myxoid Degeneration
  - Pericytomatos or Liposarcomatos Pattern
  - Many Different Malignant Mesenchymal Tumor Patterns May Be Seen.
- Type III PPB (Solid Tumors): 32% of All PPBs
  - Median Age 44 Months (range 12-147 months)
  - Dyspnea, Fever, Cough, Chest or Abdominal Pain, Pneumonia, Malaise, Anorexia
  - Pleural Effusion
  - Pathology of Type III PPB (Solid Tumor)
    - Stroma Blends with Spindle Cells Organized into Vague Fascicular Pattern of Fibrosarcoma or Pleomorphic Undifferentiated Sarcoma (MFH)
    - Stroma Resembling that Surrounding Blastema in Wilms Tumors
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    - Many Different Malignant Mesenchymal Tumor Patterns May Be Seen

- Differential Diagnosis of Type II and Type III PPBs
  - Primary or Metastatic Rhabdomyosarcoma
  - Malignant Teratoma
  - Synovial Sarcoma
  - Undifferentiated Pleomorphic Sarcoma (MFH)
  - Spindle Cell Sarcoma
  - Fibrosarcoma
  - Fibrous Histiocytoma
  - Inflammatory Myofibroblastic Tumor
  - Metastatic Wilms Tumor
- Type IR PPB (regressed PPB): 5% of All PPBs
  - Cystic Mass Similar to Type I PPB Recognized in 2008
  - Delicate Septa with No Malignant Cells
  - Small Spindle Cells Lacking Primitive Appearance
  - Foci of Dystrophic Calcifications
  - Histopathologically Distinct From CCAM/CPAMs
  - Either Regressed from Type I PPB or Precursor Lung Cyst That Mesenchyme Did Not Become Dysplastic or Malignant
  - May Present with Pneumothorax
  - Recognized in Children and Adults
- Progression of Type I to Type II to Type III: Well Recognized
  - Occurs Over Time with As Noted With Recurrences
  - Tumors Progress, but Do Not Regress from Type III to Type II or Type II to Type I
  - "Benign" Lung Cysts Followed from Infancy and Into Early Childhood May Progress from Radiologic Pure Cystic Lesion (Type I) to Type II or Type III)
- Metastatic Disease
  - Brain 15-25%
    - More Common in Type III PPBs with 54% CNS Involvement
    - Less Common in Type II PPBs with 11% CNS Involvement
  - Bone 6-10%
  - Liver 2-4%
  - Rare Sites
    - Choroid of Eye, Iris, Ovary, Adrenal Glands
    - Spinal Cord, Leptomeninges
  - Time to Metastases: 24 months from Diagnosis in Most
- Treatment
  - Surgical Excision of Type I, II and III PPBs and Lung Cysts Suspicious for PPB
  - Type I PPB with Incomplete Excision - Adjuvant Chemotherapy
  - Chemotherapy for Type II and Type III PPBs
    - Ifosfamide, Doxorubicin, Vincristine, Actinomycin D
      - Response Prompt with Maximum Response with 2-4 Courses
      - Prior Partial Resection, 2nd Look Surgery and Complete Resection after 2-4 Courses of Chemotherapy
      - Complete Response Rarely Reported and Recommend Complete Resection of Remainder of Primary Site

- Intracavitary Chemotherapy and/or Radiation Therapy With Tumor Spillage (Cis-Platinum with Systemic Chemotherapy and Radiation)
    - Intracavitary <sup>32</sup>P for Residual Pleural Disease
    - Recurrent PPB
      - Individualized Therapy
  - Radiation Therapy
    - Residual Disease After Surgery
    - Intracavitary <sup>32</sup>P for Residual Pleural Disease
  - High Dose Chemotherapy and Stem Cell Reconstitution (autologous bone marrow transplantation) In Recurrent or Metastatic PPB
    - 50% Success Rate in Limited Patients
- Surveillance in PPB
  - Lung Cysts in High Risk Children
    - Chest X-Ray Every 2 Months
    - Chest CT Every 4-6 months until 60 months of age
  - Type I PPB
    - Years 1 & 2 After Diagnosis:
      - Monthly Chest X-ray and Chest CT Every 3 months
    - 24 to 60 Months of Age: Chest CT Every 3 months
  - Type II and III PPB
    - Years 1 & 2 After Diagnosis:
      - Chest X-ray monthly
      - CT of Chest and Abdomen Every 3 Months
      - Bone Scan Every 3 Months
      - CNS/Head MRI Every 3 Months
    - Year 3 After Diagnosis
      - CT of Chest and Abdomen Every 3 Months
      - Bone Scan Every 3 Months
      - CNS/Head MRI Every 3 Months
- Five-Year Overall Survival Rates:
  - Type I PPBs: 85%
  - Type II PPBs: 58%
  - Type III PPBs: 42%
- Dicer 1 Germline Mutations in PPB Family Tumor and Dysplasia Syndrome (2009)
  - Autosomal Dominant Inheritance
  - Dicer 1 Required for Normal Branching Lung Morphogenesis
  - Dicer 1 Mutation Results in Loss of Dicer 1 Protein in Lung Epithelium in PPBs with Retention of Dicer 1 Protein in Sarcomatous/Mesenchymal Cells
  - Dicer 1 Mutant Lung Epithelium in Murine Model Leads to Impaired Branching and Cystic Dilatations Due to Lack of Epithelial-Mesenchymal Interaction
  - Postulated that Loss of Dicer 1 Function and Dysregulated Autocrine Signals from Lung Epithelium to Mesenchymal Cells Induce Cystic Formation and Lead to Malignant Transformation.
- PPB Family Tumors and Dysplasia Syndrome
  - Familial Distribution in 33%
  - Usually Occurs in First Two Decades of Life

- Associated with Dicer 1 Mutation
- Tumors/Dysplasias
  - Lung Cysts (Dicer 1 Mutation)
  - Cystic Nephroma (9-10%, Dicer 1 Mutation)
  - Wilms Tumor (Dicer 1 Mutation)
  - Dysplasias
    - Intestinal Hamartomatous Polyps (Ileal most common with intussusception)
    - Cystic Hepatic Hamartoma
  - Nasal Chondromesenchymal Hamartoma (Dicer 1 Mutation)
  - Ciliary Body Medulloepithelioma (Dicer 1 Mutation)
  - Ovarian Fibroma (Dicer 1 Mutation)
  - Childhood Cancers
    - Rhabdomyosarcoma (Dicer 1 mutation)
    - Other Sarcomas
    - Neuroblastoma, Medulloblastoma, Other CNS Tumors
    - Leukemias
    - Gonadal Tumors
      - Sertoli-Leydig Cell Tumors (Dicer 1 Mutation)
      - Dysgerminoma (Dicer 1 Mutation)
      - Seminoma (Dicer 1 Mutation)
      - Germ Cell Tumors
    - Uterine/Cervical Sarcoma Botryoides (Dicer 1 Mutation)
      - Adolescent and Young Women
    - Thyroid
      - Nodular thyroid Hyperplasia (Dicer 1 Mutation)
      - Follicular and Papillary Thyroid Carcinomas (Dicer 1 Mutation)
- Cytogenetic Findings in PPB
  - Trisomy 2 and 8
  - 17p Deletions
  - P53 Mutations
  - Rearrangements in 11p
  - Chromosomal Instability
  - CGH Amplification at 5q33-34, 11q22.2-ter, 15q25-ter, 19q11-13.2
  - CGH Gains 8q11-22.2, 20q
  - CGH Losses 9p21-24, 11p14

### **Mucoepidermoid Carcinoma (MEC)**

- Uncommon Tumor Characterized by Combination of Mucus Secreting, Squamous and Intermediate Cell Types
- Frequency of 0.1 to 0.2% of Primary Lung Tumors
- Wide Age Range from 3 to 78 Years with No Gender Bias
- Most Cases in Pediatric Age Group and Accounts for 10% of Primary Lung Tumors in This Group
- Clinical Symptoms
  - Cough, Hemoptysis, Bronchitis, Wheezing
  - Fever, Chest Pain, Clubbing of Fingers
- Clinical and Radiologic Differential Diagnosis
  - Asthma, Pneumonia, Atelectasis

- Middle Lobe Syndrome, Pleural Effusion
- Conventional Chest X-ray and CT Not Helpful in Establishing Diagnosis of Endobronchial MEC
  - Fiberoptic Bronchoscopy Usually Necessary
- Pathology Features
  - Gross Features
    - Tumor Arises in Large Airways (main and lobar bronchi, trachea)
    - Exophytic Luminal Mass: Sessile, Polypoid, Broad-Based or Pedunculated
    - Cut Surface: Gray-White-Tan with Glistening Muroid Appearance and May Have Cystic "Degeneration"
    - Variable Size: up to 6cm.
    - Dilated Bronchus With Abundant Luminal Muroid Substance in Distal Aspect
    - Adjacent Lung Atelectasis or Pneumonia
  - Histopathologic Features
    - Mucus-Secreting, Squamous and Intermediate Epithelial Cells
    - Patterns: Glandular, Tubular, Cystic, Nested and Solid
    - Close Association with Adjacent Submucosal Bronchial Glands
    - Mucus-Secreting Cells: Large Light-Blue to Gray Mucinous Cytoplasm
      - Variants: Columnar, Goblet, Cuboidal, Clear Oncocytic
    - Mucus extravasation
    - Squamous Cells: Intercellular Bridges, Usually Lack Keratin Whorls or Pearls
    - Intermediate Cells Lack Specific Differentiation -Usually Polygonal with Bland Nuclei and Abundant Amphophilic to Slightly Eosinophilic Cytoplasm
    - Calcifications and Lymphoid Aggregates
    - Grading of Bronchial MECs
      - High Grade:
        - Necrosis, Nuclear Pleomorphism
        - Mitoses, Solid or Nested Pattern
        - About 50% of Tumors
        - Infiltrate Surrounding Lung Parenchyma
      - Low Grade: Lack of Above Features
        - Typically Confined to Bronchus
        - Not Involve Adjacent Lung Parenchyma
- Molecular & Cytogenetic Features
  - t(11;19)(q21;p13): MECT1-MAML2 (Most Common Translocation [40%])
    - Disrupts Notch Signaling Pathway
    - MECT1 (MEC Translocated 1)
      - Transducer of Regulated cAMP Response Elements Binding 1 (CREB1, TORC1) and Warthin-Mucoepidermoid Tumor Translocation Partner Gene 1 (WAMPT1)
    - MAML2 (Mastermind-Like 2)
      - Encode Fusion Transcript Acts as Co-activator for cAMP Signaling Pathway
      - Linked to Prognosis in Children with MEC
  - t(1;11)(p22;q13): Cyclin D1 Located at 11q13

- Increased Cyclin D1 Expression in 20-30% of MECs
  - Multiple Translocations Most Frequently Involving
    - Chromosomes 1, 5, 7 and 11
    - Reciprocal Translocations
      - t(11;19); t(1;16); t(6;8)
      - t(3;15); t(7;15)
  - Immunophenotype
    - Negative for TTF1 and CK30
    - Positive for CK7, CK5, CK6
- Differential Diagnosis
  - Adenocarcinoma
  - Adenosquamous Carcinoma
  - Metastatic Renal cell Carcinoma
  - PEComa
- Treatment and Prognosis
  - Surgical Resection: Sleeve Resection, Segmental or Localized Resection, Lobectomy, Endoscopic Resection
  - Overall and Disease-Free Survival in Pediatrics
    - 5 Year Survival 100%
    - 10 Year Survival 100%

### **Pulmonary Carcinoid (Neuroendocrine Tumors, Well-Differentiated)**

- Although Rare in Children, Pulmonary Carcinoid Diagnosis May Be Delayed Due to Low Clinical Suspicion
- Considered As Low-Grade Neuroendocrine Carcinoma
  - Potential for Aggressive Local Growth
  - Low Potential for Metastatic Disease
- Arise from Kulchitsky Cells in Normal Basal Cell Layer of Bronchial Epithelium
  - Carcinoids Prevalence by Location
    - Foregut
      - Thymus 0.4%
      - Lung, Bronchi, Trachea 29.8%
      - Stomach 4.9%
    - Midgut
      - Small Intestine 30.4%
      - Gallbladder, Pancreas 1.0%
    - Hindgut
      - Appendix 5.1%
      - Colon 9.2%
      - Rectum 14.5%
- Endobronchial Obstructive Mass in Preadolescents and Adolescents
- Symptoms
  - Wheezing, Cough, Hemoptysis, Pneumonia, Pleuritic Pain, Dyspnea
  - Carcinoid Syndrome Rare in Absence of Metastatic Disease and Rare in Bronchial Carcinoid
  - Cushing's Syndrome in 4% (hypertension, cushingoid habitus, muscle weakness, hypokalemic alkalosis)
- Arise Within Lobar (75%) or Mainstem (10%) Bronchi & Lung Parenchyma (15%)
- Pathology Features
  - Cut Surface Firm, Homogenous Tan with Foci of Hemorrhage

- Average Size of 2-4cm
- Infiltrate Underlying Bronchial Wall and Adjacent Lung Parenchyma in "Iceberg" Pattern
- Sheets, Nests, Cords of Bland Small Cells with Finely Granular (Salt and Pepper) Chromatin, Eosinophilic Cytoplasm and Central Round Nuclei
- Tumor Cells in Background of Fine Vascular Network
- Atypical Carcinoid Associated with  $\geq 2$  mitoses per 10 HPFs
- Metastatic Disease to Regional Lymph Nodes, Liver, Bone and Brain
- Bronchial Carcinoids May Be Associated with MEN Syndromes- Most Often with Pituitary Tumors
- Genomic Alterations
  - Chromosomal Loss (Deletions): 11p (18%), 11q (36%, MEN1 at 11q13)
  - Chromosomal Gains: 5p (18%), 5q (18%), 7p (9%), 7q (9%), 9q (18%), 16q (18%), 20q (9%)
- Localized Tumor Invasion or Metastatic Disease in Pediatric Cases: 27%
- Survival with Carcinoids ( )
 

○ Series Including Adults and Children	5 Year	10 Year
▪ Typical Carcinoid	87-100%	87-93%
▪ Atypical Carcinoid	40-59%	31-59%
▪ Metastatic Disease	14-25%	NA
○ Survival in Children		
▪ Overall Survival	94%	92%
▪ Disease-Free Survival	97%	97%
- Treatment
  - Surgical Resection Treatment of Choice
    - Lobectomy/Pneumonectomy (60-75%)
    - Wedge or Segment Resection & Sleeve Resection
  - Radiation and Chemotherapy Adjuncts in Incomplete Resection, or Unresectable Tumors, Metastatic Disease or Recurrences
  - Chemotherapy with or without Radiation Response Rate of 22%
  - Molecular Targeted Agents
    - Angiogenesis (VEGF, PDGF, mTOR)
    - Bevacizumab, Everolimus, Sunitinib (Advanced Disease)

### Spindle Cell Tumors

- Congenital Peribronchial Myofibroblastic Tumor
  - Rare Benign Tumor Occurring in Fetus, Neonate and Infant
  - Other Terminologies: Congenital Mesenchymal Hamartoma of Lung, Bronchopulmonary Leiomyosarcoma, Primary Bronchopulmonary Fibrosarcoma
  - Arise from Pluripotent Mesenchyme Adjacent to Developing Bronchi at 12 weeks Gestation
  - Smooth Muscle and Cartilaginous Differentiation
  - Presents on Ultrasound In Utero or in Neonatal Period
    - Large 5-7 cm Unilateral Mass
    - Mediastinal Shift
    - Polyhydramnios, Hydrops, Respiratory Failure
  - Gross Features
    - Firm, Rubbery Mass
    - Cut Surface Yellow-Tan to Gray Whorled Surface with Fibrous Bands

- Histopathologic Features
  - Bland Spindle Cells with Large Fascicles That Surround, Displace and Distort Airways
  - Irregular Cartilage Adjacent to Entrapped Airways
  - Uniform Cellularity
- Immunohistochemistry: Diffuse Vimentin and Focal Desmin, MSA, and SMA.
- Cytogenetics: Complex Rearrangement of Chromosomes 4, 9, 10 (1 case studied)
- Electron Microscopy: Features of Myofibroblastic Origin - Spindled Cells with Dilated Rough Endoplasmic Reticulum, Infrequent Cytoplasmic Filaments, Dense Bodies and Attachment Plaques
- Treatment and Outcome
  - Complete Surgical Excision
  - Respiratory and/or Hemodynamic Compromise Due to Large Tumor Size
  - Mortality About 50% in Single Series
- Inflammatory Myofibroblastic Tumor (Inflammatory Pseudotumor, Plasma Cell Granuloma)
  - Reactive and Neoplastic Tumor Features with Slow Growth
  - Presents with Cough and/or Fever (75%) and Asymptomatic in 25%
  - Most Children >5 Years of Age
  - Radiology: Solitary, Well-Circumscribed Mass (1-12cm)
  - Location: Parenchymal (80%) or Endobronchial (20%)
  - Histopathology:
    - Proliferation of Bland Spindled Cells with Abundant Cytoplasm
    - Scattered Lymphocytes, Plasma Cells and Eosinophils
  - Immunohistochemistry: SMA, MSA, ALK1, P80 (some associated with HHV-8)
  - Electron Microscopy: Features of Myofibroblastic Origin - Spindled Cells with Dilated Rough Endoplasmic Reticulum, Infrequent Cytoplasmic Filaments, Dense Bodies and Attachment Plaques
  - Cytogenetics/Genetics:
    - Translocation of ALK with Several Partners (TPM3, TPM4, CLTC, RANBP2 [epithelioid variant-aggressive], CARS, ATIC, SEC31L1)
  - Treatment:
    - Primarily Surgical Excision
    - Local Recurrence with Incomplete Excision
    - Recurrence and Metastatic Disease May Occur: Re-excision and Oncologic Management
- Smooth Muscle Tumors
  - EBV-associated in Children with Immune Dysregulation, Auto-Immune Diseases, Immune Suppression, Solid Organ Transplantation, Primary and Secondary Immunodeficiency
  - Respiratory Tract and Gastrointestinal Tract Involvement as Single or Multifocal Tumors
  - Circumscribed Tumors with Pushing Borders
  - Spindle Cell Tumors with Variable Cellularity
  - Range from Benign to Atypical to Malignant

- EBV (EBER-1) Nuclear Reactivity in All Smooth Muscle Tumor Cells
  - CD21 Receptor Infection of Cells
- Treatment:
  - Excision of Symptomatic Tumors
  - Optimize Immune Status - Induce Regression

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## **The Role of Fiber Analysis in Asbestos Induced Lung Disease: TEM vs. SEM. Is There Controversy**

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- I. Introduction to Fiber Analysis in the Lung
- II. Producing Analytical Data, Microscopy and Analytical Procedures
- III. SEM vs. TEM pros and cons
- IV. Fiber Morphology and Disease
- V. Concluding Thoughts: SEM vs. TEM revisited

### **Introduction to Fiber Analysis in the Lung**

Occupational and environmental exposure to asbestos fibers is known to cause fibrosing and neoplastic pulmonary diseases, including asbestos airway disease, asbestosis, mesothelioma and lung cancer (1, 2). Asbestos bodies can be found in the lungs of disease free individuals, more so in industrialized areas (3). Thus, the analysis of tissue mineral fiber content and its relation to the aforementioned pathologies is of increased interest as occupational safety standards are defined and redefined as well as in the implication of a fiber source, or lack thereof, in the medico-legal context.

The different methods available for quantification of asbestos include bright field light microscopy (LM), phase contrast light microscopy (PCLM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The latter two will be a focus of this discussion.

Qualitative analysis to determine asbestos fiber type requires the coupling of electron microscopy with energy dispersive x-ray analysis (EDXA) or selected area electron diffraction (SAED). Asbestos fibers are forms of hydrated silicates which can be broadly classified into commercial amphiboles (amosite and crocidolite), noncommercial amphiboles (tremolite, actinolite and anthophyllite) and serpentine fibers (chrysotile). The most potent, in terms of pathogenicity, is crocidolite followed by amosite (4). There is little evidence to support chrysotile as a cause of mesothelioma (5, 6).

### **Producing Analytical Data**

Early work by Langer and Pooley led the way for analysis of lung tissue for mineral fiber content using ultrastructural microscopy (7-12). The technique for isolation of lung mineral fibers involves 3 main steps: 1. dissolution of organic matter (wet chemical digestion and low or high temperature plasma ashing), 2. recovery and concentration of mineral fibers, and 3. fiber content analysis by microscopy (3). Our laboratory uses the sodium hypochlorite digestion technique as has been previously described (13). As there is some variability in fiber content within the lung, generous sampling is emphasized with autopsy, pneumonectomy or lobectomy specimens providing more accurate data; however, 0.1 gram or less of wet tissue can be used. We prefer samples of lung parenchyma (one from each lobe if available) subjacent to visceral pleura and weighing approximately 0.3 grams. Areas of fibrosis or neoplasia should be avoided due to their dilutional effect on the concentration of fibers in lung parenchyma (13). Alternatively, paraffin embedded tissue may be used following a process of deparaffinization; however, a correction factor must be applied to the determined asbestos fiber concentration

(3). The recovery of fibers following digestion involves care such that no sample contamination occurs. Methods include collection/filtration onto acetate or polycarbonate filter (pore size of 0.2 -0.45  $\mu\text{m}$ ). Error through loss of fibers can be introduced if the pore filter size is too large. Filters can then be used for LM, SEM or TEM.

## **Microscopy**

There are several different microscopy methods available for analysis for lung mineral fiber content. The least complex method is conventional bright field light microscopy. Asbestos bodies can be counted on filters prepared from digested sections of lung tissue at a magnification of 200x (whole filter) or 400x (requires at least 2 asbestos bodies on two perpendicular passes at greatest diameter and requires calculation). The results are reported as asbestos bodies per gram of wet lung tissue. There is excellent interlaboratory correlation using this method (14). The down side to this form of analysis is that a large number of fibers are beyond the resolution of light microscopy and identification of fiber type is not possible (15).

PCLM has the benefit of resolving fibers with a diameter of 0.2  $\mu\text{m}$  or greater and can detect uncoated fibers, unlike light microscopy. A majority of asbestos fibers are <0.2  $\mu\text{m}$  in diameter and thus are missed using PCLM.

SEM is used by some for the quantification of tissue mineral fiber content. At low magnification (1000x) SEM can detect coated and uncoated fibers yielding results similar to that of PCLM. Higher magnifications (10-20,000x) allow for detection of fibers not visible using

PCLM. TEM is the microscopy technique preferred by many in analyzing lung fiber mineral content as it has superior resolution; detecting the smallest fibers. SEM and TEM can be coupled with energy dispersive x-ray analysis for qualitative analysis and TEM can more easily be coupled with selected area electron diffraction (SAED) for pattern analysis (3).

### **Analytical Procedures**

Counting rules of various sorts must be applied when quantitatively and qualitatively analyzing lung parenchyma for mineral fiber content. Pathogenic asbestos fibers are defined as particles with an aspect ratio of at least 3:1, parallel sides and have a length of 5 $\mu$ m or greater. This is based on prior studies which looked at fiber morphology and pathogenicity (5, 16). For our studies, using SEM, 100 fields at 1000x magnification or 200 fibers are counted, whichever comes first. The first 20 uncoated asbestos fibers and first 10 asbestos bodies are analyzed by EDXA.

Background levels of asbestos are determined by our reference population. Examination of 20 disease free control lungs between 1981 and 2001 demonstrated between 0 and 22 asbestos bodies per gram wet lung tissue as determined by light microscopy (17, 18) and <500 commercial amphibole fibers (coated and uncoated) as determined by scanning electron microscopy(3, 18). In terms of asbestosis, we use the 5th percentile of fiber content of all asbestosis cases seen at our institution as a cutoff for distinguishing a causative relation to asbestos exposure.

## **SEM versus TEM**

SEM can resolve fibers to as small as 0.3  $\mu\text{m}$  long and 0.05  $\mu\text{m}$  diameter. As pathogenic fibers are defined as  $\geq 5 \mu\text{m}$  length, this is adequate resolution for mineral fiber analysis. At 1000x asbestos bodies and uncoated fibers can be counted with similar results to phase contrast light microscopy (PCLM). Higher magnification (10-20,000x) allows for identification of fibers not visible to PCLM. SEM can be coupled with energy dispersive x-ray analysis (EDXA) for fiber type identification (3). The possibility of automation also exists though not employed. SEM lacks the ability to identify the smallest of fibers and some have criticized it as being slow (1). There is no way around electron microscopy being a time consuming and expensive process.

TEM is a widely used method for determination of mineral fiber content in tissue digestion preparations. It provides superior resolution and can be coupled with both EDXA and SAED for qualitative purposes. SAED can be helpful when 2 fibers have similar chemical composition as with talc and anthophyllite, both of which are magnesium silicates. The preanalytical process prior to TEM analysis of lung mineral fiber content is more complex and thus there is more opportunity for error through loss of fibers, contamination, and lack of a representative grid in selection for analysis.

## **Fiber Morphology and Disease**

Prior studies have proven that fibers  $\geq 5\mu\text{m}$  in length with an aspect ratio of 3:1 are pathogenic (5, 16). Studies have shown low fibrogenic and carcinogenic potential for short asbestos fibers ( $<5\mu\text{m}$  in length) (19-22). The most important aspect of fiber analysis is the

search for fibers that are pathogenic. Crocidolite and amosite (commercial amphiboles) are the most potent fibers in terms of disease causing capability. Tremolite, a noncommercial amphibole, is frequently found as a contaminant in talc, vermiculite and chrysotile. Chrysotile, the sole serpentine asbestos fiber, is easily fragmented and cleared rapidly from the lung. As mentioned previously it is frequently contaminated with amphiboles (1, 23-26). There is little evidence that pure chrysotile causes disease (23, 27, 28). Thus, if we focus our energy and attention to the short fibers we may miss longer fibers which studies have shown are more fibrogenic and carcinogenic.

### **Concluding Thoughts: SEM versus TEM Revisited**

Lung fiber burden analysis is important in the implication of a source and can help make decisions on eliminating health hazards. It is well documented in studies that amphibole fibers are the most potent in terms of fibrogenicity and carcinogenicity. Chrysotile is frequently contaminated with amphibole asbestos. There is no evidence to suggest that pure chrysotile causes mesothelioma in humans. The most important part of fiber burden is assessing the quantity and make-up of disease causing fibers. While TEM is clearly superior to SEM in resolution; being able to detect the smallest particles, these small fibers are overshadowed by the potency of larger amphiboles in terms of disease producing potency. With resolution off the table, SEM and TEM are similar in terms of their benefits. Both provide the ability to detect fibers  $\geq 0.5 \mu\text{m}$  in length with an aspect ratio of 3:1, and both can be coupled with EDXA to determine fiber composition. SEM has the ability (only with great difficulty) to provide information on crystalline structure (SAED) which becomes important when 2 fibers have a

similar chemical composition. This can be performed much more readily with TEM. Both methods are expensive and laborious. TEM has a more complex preparative process and thus there is more opportunity for fiber loss or contamination. When considering the reproducibility and precision of SEM and TEM there is considerable variability between laboratories, largely due to sample preparation (whole filter mount versus a select portion). Thus, comparison of light microscopy data is superior between laboratories (14). While both techniques are equally capable, SEM has a bias for identification of amphiboles while TEM does for chrysotile. While acknowledging that TEM has superior resolution for detecting chrysotile, several studies in human lung mineral fiber analysis have shown correlation with mesothelioma and commercial amphiboles (18, 23, 27, 30).

## SEM/TEM Revisited

SEM Pros	TEM Pros
<p>1. Resolution to 0.3 <math>\mu\text{m}</math> long and 0.05 <math>\mu\text{m}</math> diameter</p> <ul style="list-style-type: none"> <li>- At low mag (1000x) asbestos bodies and uncoated fibers can be counted with similar results to PCLM</li> <li>- At high mag (10-20,000x) identification of fibers not visible to PCLM</li> </ul>	<p>1. Preferred method by most for detection of environmental mineral fiber content*.</p> <ul style="list-style-type: none"> <li>- TEM is used by AHERA 1986</li> </ul> <p>*No method of microscopy is preferred for identification of asbestos bodies in human tissue.</p>
<p>2. Can be coupled with EDXA for qualitative analysis of fiber chemical composition</p>	<p>2. Highest resolution (to 0.5 nm) for identification of the smallest particles</p>
<p>3. Possibility for automation</p>	<p>3. Can be coupled with EDXA for qualitative analysis of fiber type</p>
	<p>4. SAED can be performed in conjunction with TEM providing information on the crystalline structure. This is helpful when 2 fiber types have similar chemical composition.</p>
SEM Cons	TEM Cons
<p>1. Lacks highest resolution capabilities</p>	<p>1. More complex preparative steps</p> <ul style="list-style-type: none"> <li>- increased opportunity for error via loss of fibers or contamination</li> </ul>
<p>2. Time consuming</p>	<p>2. Only a small portion of the filter is mounted for TEM. Is it truly representative?</p>
<p>3. Expensive</p>	<p>4. Time consuming</p>
	<p>5. Expensive</p>

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## **Electron Microprobe Analysis in Metal-Induced Lung Disease**

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### **Introduction.**

A variety of pulmonary reactions have been associated with the inhalation of metallic dusts.<sup>1,2</sup> Some of these result in familiar pneumoconioses, whereas others simply result in intrapulmonary pigment deposition. Some of the metallic materials that can cause disease as well as the typical reaction to deposits of these particulates are listed in Table 1. Electron microprobe analysis can be useful in the identification of these particulates and, in some cases, may clarify the nature of pulmonary disease. The most useful instrumentation for the identification of these particulates consists of a scanning electron microscope (SEM) equipped with a back-scattered electron (BSE) detector and an energy dispersive spectrometer (EDXA).<sup>3</sup> By means of this methodology, individual particulates may be identified and analyzed for their elemental content. The application of SEM/BSE/EDXA to the diagnosis of metal-induced lung disease is the subject of this presentation.

### **Welder's Pneumoconiosis (Siderosis).**

The inhalation of iron oxides into the lung results in the peribronchiolar and perivascular deposition of a characteristic pigment that can usually be identified by light microscopy. In the author's experience, this most commonly occurs in the setting of work as an arc welder.<sup>4,5</sup> Welding fumes consist of a variety of metal oxides, including iron, aluminum, magnesium and titanium along with various silicates and carbonates. The author has identified more than 100 cases of welder's pneumoconiosis, nearly half of which occurred as an incidental finding in an individual with lung cancer. Nearly a quarter of the cases also had asbestosis, primarily in the setting of welding in a shipyard.<sup>6</sup> Miners of hematite ore are also exposed to iron oxides along with varying amounts of silica and silicates. In addition, exposure may occur in the setting of iron smelting operations. In the latter circumstance in particular, the author has observed ferruginous bodies with black cores composed of iron oxide 'fibers'.<sup>7</sup>

Welder's pigment consists of peribronchiolar and perivascular deposition of dark brown to black iron oxide particles. These often have a characteristic golden brown halo giving them a targetoid appearance. This latter finding as well as the primarily interstitial (as compared to intra-alveolar) location permits their distinction from hemosiderin. There is usually very little reaction to the pigment, and the presence of significant fibrosis should lead to the consideration of additional exposures, such as asbestos or silica. The sheet silicates that welders are also exposed to may lead to the formation of non-asbestos

ferruginous (pseudoasbestos) bodies with broad yellow cores, which can usually be distinguished from true asbestos bodies by their light microscopic appearance.<sup>7</sup> Due to the characteristic appearance and distribution of the pigment in welder's pneumoconiosis, electron microprobe analysis is usually not required to confirm the iron composition of the particulate material.

### **Hard Metal Lung Disease.**

Formerly known as tungsten carbide pneumoconiosis, this disorder is nearly synonymous with giant cell interstitial lung disease.<sup>4</sup> Tungsten carbide is a hard metallic material that is utilized in the manufacture and application of cutting tools, drilling equipment, armaments, alloys and ceramics. It is believed that cobalt, which is used as a binder, is the causative agent of disease. Tungsten carbide per se is inert in experimental animal studies, whereas cobalt used as an abrasive for polishing diamonds has been associated with giant cell interstitial pneumonia in diamond polishers (who have no exposure to tungsten carbide).<sup>8</sup> The pathogenetic mechanism probably involves a hypersensitivity response, since fewer than 1% of exposed individuals develop interstitial lung disease. Furthermore, asthma develops in about 10% of exposed workers. Most reported cases have occurred in individuals exposed to dust during the manufacturing process or in the polishing of finished products.

Giant cell interstitial pneumonia (GIP) is an inflammatory and fibrotic reaction in which there are numerous giant cells within alveolar spaces and lining alveolar walls. Ultrastructural studies have confirmed the origin of the former from macrophages and the latter from Type II pneumocytes. This is the typical pattern in individuals with hard metal lung disease. A pattern resembling hypersensitivity pneumonitis has also been described. The finding of a GIP pattern or of interstitial lung disease in an individual with a compelling exposure history is an indication for the performance of SEM/BSE/EDXA on paraffin sections. The finding of tungsten particles with or without cobalt is considered confirmatory. Tantalum and titanium may also be identified. Although cobalt is the likely etiologic agent, it may not be identified due to its solubility in biologic fluids. Examination of bronchoalveolar lavage pellets for the characteristic multinucleate giant cells and particulate material may also be useful for confirmation of the diagnosis.<sup>9, 10</sup>

### **Berylliosis.**

Chronic beryllium disease is the classic example of a metal-induced pulmonary granulomatosis. Exposure to beryllium occurs primarily in the aerospace, electronics and nuclear power industries, where it is used in heat shields, rocket motor parts, guidance systems, optical devices, thermocouples, ceramics and crucibles. It is also used in the manufacture of dental prostheses and even some golf clubs. Exposure may also occur during the mining and extraction of beryllium ores or living in the vicinity of a

processing plant.<sup>4</sup> The development of hypersensitivity is associated with certain HLA genotypes. The granulomatous reaction seen in patients with beryllium hypersensitivity may be difficult to distinguish from sarcoidosis, and some have advocated the use of lymphocyte blast transformation assays on peripheral blood or bronchoalveolar lavage samples to assist in the differential diagnosis.<sup>11</sup>

Identification of beryllium in tissues is problematic on two accounts. As is the case for cobalt, beryllium is soluble in biological fluids, so it may be undetectable in individuals with a prolonged interval since last exposure or in tissue samples that have been in formalin for a considerable period of time. Furthermore, in the past, many energy dispersive spectrometers have used beryllium windows that would filter out the low level x-rays produced by beryllium. Other methodology that has been used to identify beryllium in tissue samples includes electron energy loss spectrometry, laser microprobe mass analysis, and secondary ion mass spectrometry.<sup>3</sup> These techniques are not available in most medical centers where such patients are likely to be seen.

The advent of thin-window detectors (with a proprietary polymeric as opposed to beryllium window) has opened up the possibility of detecting beryllium with traditional SEM/BSE/EDXA technology. Butnor et al reported such a case in which this approach was used to detect beryllium within a granuloma from a wedge lung biopsy specimen of a patient with occupational beryllium exposure.<sup>11</sup> Confirmation of this approach in additional cases is necessary before this methodology can be applied routinely.

### **Aluminosis.**

Aluminum is a lightweight metal used extensively in industrial and manufacturing processes. Exposure primarily occurs from aluminum smelting, manufacture of aluminum oxide abrasives, aluminum polishing, and aluminum arc-welding.<sup>1,5</sup> The particles often occur as fumes, with sizes ranging from 0.1 to 1.0  $\mu\text{m}$ .

Pulmonary disease from aluminum exposure is uncommon. In some cases, all that is found is perivascular and peribronchiolar deposits of aluminum particles within macrophages. The dust has a gray-brown granular appearance and is refractile.<sup>5</sup> Disease resulting from exposure to aluminum oxide abrasives (corundum) has been referred to as Shaver's disease. Corundum consists of aluminum oxide, silica, ferric oxide and traces of titanium.<sup>4</sup> In fatal cases of Shaver's disease, the lungs are heavy, grayish black, and have dense fibrotic areas scattered throughout, dense pleural adhesions, and subpleural emphysematous bullae. The latter may give rise to spontaneous pneumothorax.

Fibrotic lung disease may also occur as a result of exposure to aluminum among potroom workers (aluminum smelting) or aluminum arc-welders.<sup>12,13</sup> The fibrotic areas in the lung and regional lymph nodes may have a metallic sheen. Other reactions that have been described among individuals exposed to aluminum dusts include granulomatous reaction resembling sarcoidosis, desquamative interstitial pneumonia and alveolar proteinosis.<sup>5,14,15</sup> Analysis by EDXA in these conditions typically demonstrates

particles with a peak for aluminum only. The rarity of these conditions suggests that there may be a hypersensitivity component to the response, similar to that of beryllium or cobalt.<sup>1</sup>

### **Zirconium Lung Disease.**

Zirconium is a grayish metal used in the production of steel, refractory ceramics manufacture, enamels and glasses, as a polishing and abrasive agent, and as a substitute for sand in foundries.<sup>1</sup> Exposure occurs during the mining or refining of zirconium ore, or in any of the applications noted above. There is generally little pathologic reaction to the accumulation of dust within macrophages in a perivascular or peribronchiolar location. In rare cases, pulmonary fibrosis has been described.<sup>16</sup> The occurrence of a granulomatous reaction has been suggested but is controversial and unproven.<sup>17</sup> Analysis by EDXA demonstrates particles with peaks for zirconium (Zr).

### **Rare Earth Pneumoconiosis.**

Rare earths include cerium, lanthanum, neodymium and samarium. They typically occur as the oxides and exposures come from processing of rare earth ores, manufacture or use of carbon arc lamps, and manufacture or use of cerium oxide rouge used for the polishing of glass lenses.<sup>5</sup> Disease from exposure to rare earths is very uncommon with only about 20 cases having been reported. Reactions include pulmonary fibrosis and granulomatous disease.<sup>18</sup> Analysis by EDXA primarily demonstrates peaks for cerium although trace levels of other rare earths may be detected as well. Cerium oxide is birefringent with polarizing microscopy.

### **Silicon Carbide Pneumoconiosis.**

Silicon carbide (carborundum) is a synthetic abrasive widely used because of its hardness. It is used for abrasive wheels and in the manufacture of refractory materials for boilers and foundry furnaces.<sup>4</sup> Silicon carbide is thought to be inert, and only one case with pathologic findings has been described. This showed perivascular and peribronchiolar as well as intraalveolar deposits of pigmented dust with occasional ferruginous bodies formed on silicon carbide 'whiskers'.<sup>19</sup> Analysis of dust recovered from lung tissue by x-ray diffraction demonstrated the presence of predominantly silicon carbide with minor traces of silica and tungsten carbide.

## **Cadmium-Induced Lung Disease.**

Cadmium is used in the manufacture of alloys and alkaline accumulators and in the control of atomic reactors.<sup>4</sup> Chronic exposure to cadmium results in emphysematous changes. Diagnosis requires a careful occupational exposure history or analysis of lung tissue for cadmium.<sup>20</sup> Cadmium also occurs in cigarette smoke, which is the most common cause of emphysema.

## **Other Metals.**

A variety of other metals (Table 1) may accumulate in the lungs with little or no reaction. Some of these are named pneumoconioses (stannosis for tin, baritosis for barium) while others are not. Exposures to these metals manifest as peribronchiolar and perivascular deposits of pigment. Specific identification of these pigment deposits by EDXA is seldom required since there is no clinical symptomatology or impairment associated with the exposures, although they may produce interstitial markings on chest roentgenograms due to impedance of the x-rays by the high atomic number dusts. Exposures to very high levels of metal fumes can cause metal fume fever, which may be associated with a chemical pneumonitis that microscopically has the appearance of diffuse alveolar damage.<sup>4</sup> Finally, it should be noted that metals may be a component of mixed dust pneumoconiosis.<sup>21</sup>

## **Dental Technicians' Pneumoconiosis**

Dental laboratory workers may be exposed to a variety of different particles. Prosthetic devices made of metal alloys are polished with high-speed abrasive wheels which may generate dust composed of silica or silicon carbide. Asbestos molds are used in the process of dental gold casting, and dismantling of the molds may result in substantial exposure to aerosolized asbestos fibers.<sup>4</sup> Furthermore, analysis of lung tissue from some dental technicians has demonstrated the presence of chromium-cobalt-molybdenum alloys which may be a factor in the development of pneumoconiosis.<sup>22</sup> These alloys may cleave into elongated fragments that can be coated with iron to form ferruginous bodies. Dental technicians may also be exposed to acrylic resins (used in the preparation of dental prostheses) or alginate impression powder, which may contribute to the development of pneumoconiosis. The pathologic response is interstitial fibrosis. In cases with significant exposure to silica, silicotic nodules or progressive massive fibrosis may be observed.<sup>4</sup>

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**Table 1. Metal-Induced Lung Disease**

<u>Agent</u>	<u>Disease</u>	<u>Reaction</u>
Iron oxide	Welder's pneumoconiosis Siderosis	Perivascular/peribronchiolar pigment
Tungsten carbide	Hard metal lung disease	Giant cell interstitial pneumonia
Beryllium	Berylliosis	Granulomatous inflammation
Aluminum	Aluminosis	Interstitial fibrosis, granulomas
Zirconium oxide	Zirconium lung disease	Granulomatous inflammation
Rare earths	Rare earth pneumoconiosis	Interstitial fibrosis, granulomas
Silicon carbide (SiC)	SiC pneumoconiosis	Interstitial fibrosis
Cadmium	Emphysema	Bullous emphysema
Tin dioxide	Stannosis	Perivascular/peribronchiolar pigment
Barium sulfate	Baritosis	Perivascular/peribronchiolar pigment
Antimony	Antimony lung disease	Perivascular/peribronchiolar pigment
Others*	No specific name	Perivascular/peribronchiolar pigment

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\*Includes chromium, copper, nickel, titanium and zinc

# USCAP

2011 ANNUAL MEETING



February 26-March 4, 2011

Henry B. Gonzalez Convention Center  
San Antonio, TX

## **Society for Ultrastructural Pathology**

**Sunday, 2/27/11; 8:30 a.m. - noon**

**Ultrastructural Pathology of Pulmonary Corpora Amylacea**

**Samuel P. Hammar, MD**

**Diagnostic Specialties Laboratory**

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My interest in corpora amylacea was somewhat serendipitous in that over a period of years I started seeing a significant number of cases primarily in patients who had mesothelioma. From the information provided in the 3<sup>rd</sup> edition of Dail & Hammar's Pulmonary Pathology, corpora amylacea are seen in autopsy specimens somewhere between about 0.6 to 2.8%. These structures are pink in H&E stained sections and are usually seen in alveolar spaces of the lung. There is no specific location in which they are found with respect to lobe or specific region of the lung. By light microscopy, one can see that they are made up of radiating fibrillar lines with delicate circumferential lines. They vary between 30 to 200  $\mu\text{m}$ . They are composed of glycoprotein and are free of lipid, calcium, and iron. Little of their substance is stated to be altered by tissue processing, unlike pulmonary alveolar microlithiasis or blue bodies. The structures stain blue on Mason's trichrome stain; magenta on PAS stains; and bluish-green on Alcian blue stains. They also stain slightly pinkish with mucicarmine, however, most of the time are negative. They are not always spherical. Some are elliptical and some are rectangular. As described in the 3<sup>rd</sup> edition of Dail & Hammar, there are frequent inclusions in the center of these bodies that range from black fragments that are spherical to straight black fragments that might be carbon. What has been unique in my experience is the presence of asbestos bodies in some cases within the center or near the center. I have also seen asbestos fibers in the center of these structures. It has been suggested by some that corpora amylacea are similar to asbestos body formation in that in some way some type of carbohydrate material encloses foreign material. I have seen many different structures, including iron and other particulates, in the corpora amylacea and have come to conclude that pulmonary corpora amylacea rid the lung of foreign material.

Prior to looking at corpora amylacea by electron microscopy, I reviewed a number of books and articles and could not find any ultrastructural morphology of pulmonary corpora amylacea. The ultrastructural appearance of pulmonary corpora amylacea is

very interesting and does not jump out as being something I have seen. Perhaps it should have been one of the “what izzits” that we had on the walls in our 1982 meeting in Seattle for the Society of Ultrastructural Pathology.

I have tried to figure out exactly how pulmonary corpora amylacea form. One thing that is characteristic of pulmonary corpora amylacea is that in almost every case, if you have a good tissue section, there are macrophages that are surrounding the corpora amylacea. In one of the first cases I observed, I thought I could see fibrillar material inside the macrophage, although I was never certain. I have kept looking and so far I have not concluded that the macrophages actually produce the material corpora amylacea are made from, but from a practical point-of-view, if you look at asbestos bodies, then it is highly likely that macrophages are the source of them. As stated, I think these structures are very common in people who have mesothelioma because they are exposed to dust, probably a mechanism by which the body gets rid of foreign material such as asbestos fibers, etc. As far as I know, corpora amylacea do not cause any specific disease.

I have looked at the ultrastructural appearance of edema fluid in patients who have pulmonary edema and the edema fluid does not look like the type of material that corpora amylacea is made up of, although who knows how a type of material can be altered over a period of time. I have also compared the ultrastructural appearance of prostatic corpora amylacea to those in the lung and they are entirely different. The corpora amylacea in the prostate do not have the radiating fibrillar lines or delicate circumferential lines. Also, it is my experience that corpora amylacea in the prostate are usually found in prostatic gland lumens and do not show macrophages around them. Perhaps, however, I have not looked at enough cases of prostatic corpora amylacea. I have also looked in the “bible” (Ghadially’s Ultrastructural Pathology of the Cell and Matrix, 4<sup>th</sup> Edition). Dr. Ghadially refers to corpora amylacea that he shows in his book as “polyglucosan bodies” (corpora amylacea, Laflora’s bodies, Lafora-like bodies,

Bielschowsky's bodies, and amylopectin bodies). Dr. Ghadially describes these polyglucosan bodies as rounded, oval, or elongated bodies composed of short filaments (about 8 nm thick), electron dense particles, and electron dense amorphous material. Dr. Ghadially states these components are usually homogeneously distributed, but at times the amorphous material forms an electron dense core. The particles are often deployed along the filaments so that a beaded filament appearance is produced. Some authors speak about branching of filaments, but one cannot be certain of this by just looking at routine electron micrographs. The polyglucosan bodies (corpora amylacea bodies) shown in Dr. Ghadially's book do not have the same morphology as pulmonary corpora amylacea, since the ones he refers to in his book are in the central nervous system.

At this point in time, I think pulmonary corpora amylacea are structures that are there to remove foreign material that is probably larger than what macrophages can remove.

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