The role of Electron Microscopy in the study of cytologic specimens.
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Introduction

The field of Cytology is divided into exfoliative cytology and fine needle aspiration (FNA). Cytology can be practiced at different levels. One of these requires only of a needle, syringe, Papanicolaou and Diff Quik stains and a light microscope. A few histochemical stains can also be used, at least mucicarmine, PAS and others. 70 – 80% of cytologic diagnoses can be made in this manner. There are times however, when the diagnostic capabilities fall short and perhaps only a diagnosis of malignant, favor epithelial neoplasm or malignant neoplasm can be rendered. It all depends upon the degree of sophistication and accuracy desired. One step further is by making use of the many antibodies available in Immunohistochemistry (IHC). By IHC, another 20 % can be resolved. But in a number of these, the diagnosis can be refined, not totally resolved. In order for cytology to be practiced in a “no nonsense” fashion the diagnoses must be rapid and as precise as possible. The use of ancillary techniques are required for cytologic diagnoses to be firm and reliable. For reliable, specific cytologic diagnoses, we have found EM to be the technique that solves the most difficult problems, is the most cost-effective and efficient when the diagnostic cells are present in the sample.

Fine needle Aspiration (FNA) has changed the practice of Cytopathology. But in order to practice cytology effectively one needs: knowledge of the criteria, effective use of ancillary techniques and experience in the diagnostic techniques chosen. The more specific the diagnosis attempted, the more are ancillary methods required, in particular EM where the subcellular elements can be visualized providing sound morphological support to the diagnosis.

There are pitfalls in FNA diagnoses: False negatives due to improper sampling as a result of performing few passes of the needle, the lesion being too deep, or the sample not being representative of the lesion.

Limitations to the practice of cytology: There are many “look –alikes”. These are cells that are difficult to distinguish from the ones of other tumors/ tumor types and /or from other organs. Although there are published criteria that aids us to make cytologic diagnoses and there are entities for which the criteria can help us make an unequivocal diagnosis in the proper clinical
context such as papillary carcinoma of the thyroid when intranuclear pseudoinclusions are present in a thyroid aspirate. However, there are many cytologic entities which we encounter on a daily basis that share cytologic characteristics with other entities. It is because of the ‘look-alikes’ that ancillary techniques are necessary. The size of most cytologic samples is many times limited and in some cases consists of only a handful of cells. The absence of “patterns” that are so helpful in the practice of surgical pathology represent a drawback associated with the practice of cytopathology(only seldom present in cell blocks).

IHC is the most commonly used ancillary technique due to the fact that it does not require expensive equipment or additional expertise to interpret the findings. IHC has many advantages and many drawbacks as well, in particular in the practice of cytology. Cytologic samples are more difficult to interpret since there are problems with background and non specific staining given the small, often bloody samples. IHC obtains better results on cell blocks and in many cases the cell block may not contain diagnostic material. IHC can be performed on smears but few technologists obtain good results.

On the other hand electron microscopy (EM) is more appropriate for tiny samples. As long as a small group of cells is available for ultrastructural evaluation, and these are preserved, one can often successfully make a definitive diagnosis. However, ultrastructural preservation is paramount. The ancillary techniques available to the field of cytology are the same ones used in surgical pathology: Histochemistry (HC), IHC, flow cytometry, molecular biology and EM. We believe that EM is the most helpful on many occasions, although we make use of all techniques available as they are applicable to our diagnostic needs.

Electron Microscopy at the present time is going through a period in which its value is overlooked. For the practice of cytology, its usefulness is unparalleled.

**Everyday diagnostic problems in the practice of cytology in which EM is necessary:**

1- Pleural fluids with markedly atypical cells
   A- Reactive mesothelial cells(atypical mesothelial cells) For reactive vs malignant mesothelial cells EM not helpful.
   B- Metastatic carcinoma

2- Liver masses
   A- Primary neoplasm
      1- Hepatocellular carcinoma
      2- Cholangiocarcinoma(less common)
   B- Metastatic carcinoma

3- Lung masses
   A- Primary
B- Metastatic

4- Abdominal/ pelvic masses- often involving several organs( ? primary)? which of the involved organs
5- Retroperitoneal masses
   A. Lymphoid
   B. Metastatic
   C. Neural
   D. Germ cell

6- Lymph node aspirates
   A- Lymphoma
   B- Metastatic carcinoma
      1-Known primary
      2-Unknown primary

7- Renal mass- type
   A. Clear cell
   B. Other types( Medullary carcinoma)

8- Lymphoma vs small cell carcinoma vs melanoma( anywhere)

9- Aspirate in patients with one or more previous primaries.
   A. A new primary
   B. Which one of the previous primaries

What are those ultrastructural findings that help us categorize tumors even with limited EM findings?

1- Glandular or not, Microvilli, length and type.
   A- Length and type
      1- If microvilli are short and stubby, with anchoring filaments. This places the tumor in the broad category of the gut.
      2- If long and sinuous, branching- mesothelial, also in pancreatic and ovarian tumors.
   B- Microvilli associated with glycocalyx with anchoring filaments, probably colon or small bowel
   C- True microvilli or filopodia
2- Carcinoma or not
3-Cell junctions, well developed desmosomes

1-Well formed
2- Primitive
3- Neuroendocrine or not

4-Membrane bound vs lysosomes

5- Glycogen
   A-Clear cell neoplasms
   B-Germ cells
5-Filaments

6-Melanoma
   A-Melanosomes
   B-Other structures that appear similar

It is important to keep in mind that EM has multiple uses in the diagnostic arena. Electron Microscopy can: Narrow the differential diagnosis, establish the organ or organ system of origin, help by confirming IHC results (in this role it is a great adjunct to IHC), clarify light microscopy and it is crucial to note that EM can make a firm diagnosis. It is the technique of choice in many cases where making a precise diagnosis is imperative for patients to obtain the precise therapy or be placed in a particular therapeutic protocol, as in inoperable cases

It is imperative to keep in mind that the ultrastructural diagnosis of cytologic samples has to be rendered utilizing in many cases only the most essential or minimal criteria. This fact may cause rare cases to be missed or misinterpreted since uncommon types of cases may share these “most basic” ultrastructural features. However, even with this caveat, EM assists and confirms the cytologic diagnoses more than any other ancillary technique by itself. Combining IHC with EM and any other diagnostic modality, as applicable, can improve diagnostic accuracy.

Conclusions

• Careful selection of the appropriate ancillary technique to solve the diagnostic problem at hand is paramount.

• IHC is the technique of choice when a few antibodies (3-4 antibodies) can solve the diagnostic problem.
• EM is the technique of choice when a large battery of antibodies would be necessary to solve a diagnostic problem or when IHC is inconclusive.

• Understanding the limitations of the ancillary technique of choice is essential.

• Favoring a technique to the exclusion of others places pathologists at a disadvantage and may result in the wrong diagnosis.

• A judicious combination of ancillary techniques is always the best approach.

• Electron microscopy may make a diagnosis not even suspected.
Ultrastructural Evaluation of Peripheral Blood and Fibroblast Cultures in Diagnosis of Metabolic Storage Disorders

Society for Ultrastructural Pathology Companion Meeting
USCAP, Baltimore MD
March 3, 2013

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Professor of Pathology, Immunology and Pediatrics
Department of Pathology and Immunology
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Department of Pediatrics
Texas Children’s Hospital and Baylor College of Medicine
Houston Tx
Lysosomal Storage Diseases in Children

- Rare Diseases – 1 in 1,500 to 7,000 Births
- Inherited Deficiency in 1 or More Catabolic Lysosomal Enzymes
- More Than 50 Different Disorders Characterized by Accumulation of Specific Substrate
- Many Steps Necessary for Synthesis and Processing of Lysosomal Enzymes – Prone to Defects and Dysfunction

- Neonatal Presentations
  - Sphingolipid Disorders: Fabry, Farber, Gaucher, Krabbe, GM 1 Gangliosidosis, Niemann-Pick Type A
  - Mucopolysaccharidosis Disorders: MPS I Hurler, MPS IVA Marquio, MPS VII Sly
  - Glycogen Storage Disease: GSD II Pompe
  - Glycoprotein Storage Disease: Sialidosis Type I & II, Mucolipidosis Type I, Schindler Disease
  - Transport & Trafficking Disorder – ISSD, Sialuria, Salla Disease, Niemann-Pick Type C
  - Multienzyme Defect – Galactosialidosis, I-Cell, Mucolipidosis Type 2, Multiple Sulfatase Deficiency, Prosaposin Deficiency
Gaucher Disease
- Most Common Lysosomal Storage Disease – 1 in 75,000 Births; Ashkenazi Jews 1 in 1,000 Births
- Autosomal Recessive Inheritance
- Three Types Based on Clinical Signs & Symptoms
- Diagnosis Based on Identification of Gaucher Cells in Peripheral Blood or Bone Marrow; Beta-Glucocerebrosidase Activity in Leukocytes; or Molecular Analysis for Mutation in Glucocerebrosidase Gene (GBA, 1q21)

GBA Gene (1q21)
- Point Mutation N370S (75% Ashkenazi Jews; 30% non-Jews)
- Frame Shift 84GG (Jews)
- Mutation L444P: Swedish Norrbottilians
- Single Progenitor Ashkenazi for N370S & 84GG

<table>
<thead>
<tr>
<th>Gaucher disease classification</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3a</th>
<th>Type 3b</th>
<th>Type 3c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>Childhood to adult</td>
<td>First year</td>
<td>Childhood</td>
<td>Childhood</td>
<td>Childhood</td>
</tr>
<tr>
<td>Hematologic</td>
<td>Anemia, thrombocytopenia</td>
<td>Minimal thrombocytopenia</td>
<td>Anemia</td>
<td>Extensive</td>
<td>Minimal</td>
</tr>
<tr>
<td>Skeletal</td>
<td>Osteopenia, osteosclerosis</td>
<td>Minimal</td>
<td>Osteopenia, osteosclerosis</td>
<td>Severe</td>
<td>Minimal</td>
</tr>
<tr>
<td>Neurologic</td>
<td>None</td>
<td>Generalized seizures, hypertonia, profound mental retardation, apnea</td>
<td>Myoclonus, progressive dementia, ataxia, and myoclonus</td>
<td>Supranuclear gaze palsy</td>
<td>Supranuclear gaze palsy</td>
</tr>
<tr>
<td>Other systems</td>
<td>Hepatic fibrosis, pulmonary hypertension, lymphoma</td>
<td>Congenital ichthyosis</td>
<td></td>
<td></td>
<td>Cardiac and vascular calcifications</td>
</tr>
<tr>
<td>Progression</td>
<td>Slow</td>
<td>Rapid</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Life span</td>
<td>Shortened, may be normal</td>
<td>Death before 2 years of age</td>
<td>20-30 years</td>
<td>Shortened</td>
<td>Shortened</td>
</tr>
<tr>
<td>Mutation association</td>
<td>N370S</td>
<td>Diverse</td>
<td>L444P</td>
<td>Diverse</td>
<td>D409H</td>
</tr>
<tr>
<td>Ethnic predilection</td>
<td>100 times more common in Ashkenazi Jews</td>
<td>None</td>
<td>Norrbottian region, Sweden</td>
<td>None</td>
<td>Spanish?</td>
</tr>
</tbody>
</table>
### TABLE 1  Lysosomal Disorders Associated with Hydrops Fetalis

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2 Gaucher disease</td>
<td>Glucocerebrosidase (acid β-glucosidase)</td>
</tr>
<tr>
<td>Galactosiadosis</td>
<td>β-galactosidase protective protein (PPCA)</td>
</tr>
<tr>
<td>GM1 gangliosidosis</td>
<td>β-galactosidase</td>
</tr>
<tr>
<td>Sialidosis</td>
<td>Neuraminidase (sialidase)</td>
</tr>
<tr>
<td>Mucopolysaccharidosis I</td>
<td>α-iduronidase</td>
</tr>
<tr>
<td>Mucopolysaccharidosis IVA</td>
<td>Galactose-6-sulfatase</td>
</tr>
<tr>
<td>Mucopolysaccharidosis VII</td>
<td>β-glucuronidase</td>
</tr>
<tr>
<td>Mucolipidosis II (1-cell disease)</td>
<td>N-acetylglucosamine 1-phosphotransferase</td>
</tr>
<tr>
<td>Niemann-Pick disease type A</td>
<td>Sphingomyelinase</td>
</tr>
<tr>
<td>Niemann-Pick disease type C</td>
<td>NPC-1 protein</td>
</tr>
<tr>
<td>Multiple sulfatase deficiency</td>
<td>Multiple sulfatase enzyme</td>
</tr>
<tr>
<td>Farber disease</td>
<td>Acid ceramidase</td>
</tr>
<tr>
<td>Wolman disease</td>
<td>Acid lipase</td>
</tr>
<tr>
<td>Infantile free sialic acid storage disease</td>
<td>Sialic acid transporter</td>
</tr>
</tbody>
</table>
Therapy for Gaucher Disease: Imiglucerase (Cerezyme: substitute for Glucocerebrosidase)

Table III. Children and adolescents (<18 years) with Gaucher disease: risk assessment and dose

<table>
<thead>
<tr>
<th>Criteria</th>
<th>High risk (at least one of the following)</th>
<th>Low risk (all of the following criteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial dose Criteria</td>
<td>60 IU/kg every 2 weeks</td>
<td>30 IU/kg every 2 weeks</td>
</tr>
<tr>
<td>Glucose, Hemoglobin 2 g/dL; below lower normal limit for sex and age</td>
<td>Platelets &gt;60,000/mm² or documented abnormal bleeding</td>
<td>Hemoglobin maximum of 2 g/dL; below lower normal limit for sex and age</td>
</tr>
<tr>
<td>Delayed growth</td>
<td>Bone disease limited to osteopenia and marrow infiltration</td>
<td>Platelets &gt;60,000/mm² on 3 measurements</td>
</tr>
<tr>
<td>Active bone disease</td>
<td>Hepatic volume &gt;2.5 times normal value</td>
<td>Volumetric increase in spleen &gt;15 times normal value</td>
</tr>
<tr>
<td>Alterations in hepatic function or volumetric increase &gt;2.5 times normal value</td>
<td>Spleen volume &lt;15 times normal value</td>
<td>Normal hepatic, cardiac, pulmonary, and renal functions</td>
</tr>
<tr>
<td>Pulmonary alterations</td>
<td>Kidney disease</td>
<td></td>
</tr>
</tbody>
</table>

Table V. Therapeutic goals in ERT

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>At 1 year</th>
<th>At 2 years</th>
<th>After 5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin concentration</td>
<td>Normal levels should be reached in most patients</td>
<td>Normal levels should be maintained</td>
<td></td>
</tr>
<tr>
<td>Greater increase in more severe anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>Normal levels should be maintained (may not reach normal values)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsplenec tomized patients with thrombocytopenia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60,000/mm³</td>
<td>Should increase 1.5- to 2-fold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60,000/mm³</td>
<td>Should increase 1.5-fold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenectomized patients with thrombocytopenia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;120,000/mm³</td>
<td>Increases should be progressive and continuous (may not reach normal values)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic volume&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Increases should be progressive and continuous (may not reach normal values)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen volume&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Should decrease by 30%-40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone disease</td>
<td>Should decrease by 50%-60%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain and bone crisis</td>
<td>Improvement in bone mineral density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction or remission in bone pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission of bone crises</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevention of osteonecrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Niemann-Pick Disease
- Group of Sphingomyelin-Cholesterol Lipidoses
- Autosomal Recessive Disorders Associated With Hepatosplenomegaly, Variable Neurological Deficits, Interstitial Lung Disease & Storage of Sphingomyelin
- Deficiency of Isoelectric Forms of Sphingomyelinase with 6 Types Characterized:
  - Type A through F
- Type A: Infantile Form in 1st Year of Life
  - Hepatosplenomegaly and CNS Degeneration
  - Rapid & Progressive Loss of Neurologic Function
  - Interstitial Lung Disease & Macular Cherry Red Spot
  - Death in 2 to 3 Years
- Type B: Non-Neuropathic Less Severe Than Type A with Survival Into Adulthood (thrombocytopenia)
  - Types A & B: Incidence 1; 250,000 (Ashkenazi Jews Gene Frequency 1:100)
  - Sphingomyelin Phosphodiesterase-1 (SMAD-1, 11p15) Mutation
- Type C: Presents in Infants, Children & Adults
  - Prevalence of 1:150,000
  - Middle to Late Childhood Form After Normal Development
    - Clumsiness, Gait Problems Progressing to Ataxia, Decreased Cognition & Vertical Supranuclear Ophthalmoplegia, Seizures, Dysphagia, Dysarthria, Dystonia & Hepatosplenomegaly
  - Neonatal Form With Hepatic Infiltration, Prolonged Jaundice, Fetal Ascites in Some, & Pulmonary Infiltrate
  - Infantile Form with Hypotonia & Developmental Delay
- Type C: Adult Form
  - Ataxia, Supranuclear Ophthalmoplegia & Cognitive Impairment
  - Slower Progression than Infantile or Childhood Form
  - Subtle Signs and Symptoms in Childhood in Some: Hepatosplenomegaly, Learning Difficulty, Deafness, Impaired Vertical Gaze
  - Dementia, Depression & Bipolar Disease or Schizophrenia May be Only Symptoms in Some Adults
- Genetics
  - NPC1 (18q11-12) in 90% of Type C Cases
    - Membrane Glycoprotein: Intracellular Trafficking of LDL Cholesterol
- NPC2 (14q24.3, HE1) in 4% of Type C Cases
  - Soluble Lysosomal Protein (Cholesterol-Binding Protein)

- Type D: Nova Scotia Ancestry
  - Similar Course as Type C Cases
  - Neurologic Involvement
  - Both Types C & D: Normal Levels of Sphingomyelinase

- Type E: Indeterminate Form in Adults
  - Closely Related to Type C
  - No Neurologic Involvement

- Type F: Childhood Onset of Splenomegaly
  - Lack Neurologic Involvement
  - Decreased Activity of Heat-Labile Sphingomyelinase
  - Sea-Foam Histiocytes

- Management
  - No Treatment That Modifies Onset, or Neurologic Progression or Prolongs Lifespan

- Experimental Approaches Under Study
  - Stem Cell Transplant – Not Effective
  - In Utero Stem Cell Transplant – Transitory Effect
  - Retroviral Mediated Transfer of Sphingomyelinase Increases Enzyme by 16-fold in Cell Cultures
  - Direct Intracerebral Transplantation of Neural Progenitor Cells Increases Enzyme by 5-fold
  - Treatment with Tamoxifen & Vitamin D Reduces Oxidative Damage
  - Miglustat Inhibits Glycosphingolipid Biosynthesis, Reduces Lipid Storage, Improves Endosomal Uptake and Normalizes Lipid Trafficking in B Lymphocytes
**Neuronal Ceroid Lipofuscinosis (Batten Disease)**

- Inherited Lysosomal Storage Disease
- Autosomal Recessive (all but 1 form)
- Most Common Pediatric Progressive Encephalopathy
- Incidence in USA: 1:12,500
- Congenital, Infantile, Late Infantile, Juvenile and Adult Forms
- Childhood Form: Progressive Loss of Vision, Mental and Motor Deterioration, Seizures and Premature Death
- Rare Adult-Onset Form: Dementia

- Pathomorphologic Characteristic:
  - Autofluorescent, PAS-Positive, Sudan Black Positive Granules
  - Granules Resistant to Lipid Solvents
  - Granules Accumulate in Most Nerve Cells and to Lesser Extent in Many Cell Types (Leukocytes)

### Age at Manifestation vs. Mutated Genes

<table>
<thead>
<tr>
<th>Age at Manifestation</th>
<th>Mutated Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth</td>
<td>CLN10, CLN1</td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>CLN1, CLN2, CLN1, CLN5, CLN6, CLN7, CLN8</td>
</tr>
<tr>
<td>2–4 years</td>
<td>CLN3, CLN1, CLN8, CLN10, CLN9</td>
</tr>
<tr>
<td>School age</td>
<td>CLN1, CLN10, CLN4</td>
</tr>
<tr>
<td>Adulthood</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transmembrane Protein (ER)</th>
<th>SCMAS</th>
<th>Age at Manifestation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLN6</td>
<td></td>
<td></td>
<td>Late infantile</td>
<td>Similar to other late infantile variations, with slightly later onset</td>
</tr>
<tr>
<td>CLN7</td>
<td>MFSD8 channel (Siitola et al. 2007)</td>
<td>Membrane Protein</td>
<td>SCMAS</td>
<td>Late infantile (variant)</td>
</tr>
<tr>
<td>CLN8</td>
<td>CLN8 (Ranta et al. 1999)</td>
<td>Transmembrane Protein (ER)</td>
<td>SCMAS</td>
<td>Late infantile (variant)</td>
</tr>
<tr>
<td>CLN9</td>
<td>CLN9 (Schulz et al. 2004)</td>
<td>Unknown</td>
<td>SCMAS</td>
<td>Juvenile</td>
</tr>
<tr>
<td>CTSD</td>
<td>Cathespain D (Siitola et al. 2006b)</td>
<td>Soluble Enzyme</td>
<td>SAPs</td>
<td>Congenital and Late infantile</td>
</tr>
<tr>
<td>Unknown</td>
<td>Unknown (Siitola et al. 2006a)</td>
<td>Unknown</td>
<td>SAPs</td>
<td>Adult</td>
</tr>
</tbody>
</table>
Therapy for Neuronal Ceroid Lipofuscinosis

- Chaperone Therapy: Provide Structure for Re-Folding to Produce Active Enzyme
- Substrate Reduction Therapy
- Systemic Enzyme Replacement Therapy
- Convection-Enhanced Protein Delivery in Solution
- Stem Cell Transplant (BMT, Umbilical Cord Blood, Neural Stem Cells)
- Direct CNS Vector-Mediated Gene Therapy
I. Asbestos

Asbestos is the generic term typically used for six naturally occurring fibrous silicates that are or have been used as insulation in many industries because of their thermal and chemical stability, high flexibility, tensile strength, and low electrical conductivity. Asbestos fibers are classified as either serpentine or amphibole. The vast majority of asbestos in use in North America is serpentine chrysotile asbestos [1]. Amosite and crocidolite are the predominantly used amphibole fibers (commercial amphiboles), while tremolite, actinolite, and anthophyllite have had limited usage (they are often referred to as “noncommercial” amphiboles) [2]. Asbestos has not been mined in the United States since 2002, therefore all asbestos used in manufacturing is imported. All the asbestos currently used in the United States is chrysotile. In 2011, the vast majority of this chrysotile asbestos was imported from Canada (>90%), while the
remainder came from Brazil and Zimbabwe [3]. In the year 2011, the United States imported an estimated 1100 metric tons of asbestos, a more than 90% decrease since the year 2000 [3]. Approximately 60% of asbestos is used for roofing products, while all other materials, including gaskets and friction products, account for the remainder.

Asbestos bodies are asbestos fibers that have become coated with iron by macrophages [4]. In animal models this happens as early as two months after exposure, and this time course also seems to be a reasonable estimate for humans [5, 6].

II. Asbestos-Related Diseases

Asbestos becomes a health hazard when it is inhaled. Inhaled asbestos fibers are 20 to 100 times as long as they are wide. Whether fibers are inhaled, and which areas in the lung they reach depends more on their diameter than on their length. Fibers longer than 100 µm are usually trapped in the nose, while fibers between 40 µm and 100 µm often end up lodged in the tracheobronchial tree. Even shorter fibers can enter the peripheral airways or alveoli. Thus, the mean length of asbestos bodies found in lungs is approximately 35 µm with a 2-5 µm diameter [7, 8].

There are four main categories of asbestos-related diseases:

1. Non-malignant pleural disease
2. Asbestosis
3. Asbestos-related lung cancer
4. Mesothelioma

Exact prevalence rates are difficult to find, probably because conditions other than mesothelioma are inconsistently reported and assigning asbestos as a cause is problematic
in many cases. A recent publication highlights the frequencies with which asbestos-related diseases are encountered [9].

**Pleural plaques** are found in 3 to 58 percent of people exposed to asbestos, compared to 0.5 to 8 percent in the general population. Although pleural plaques are commonly considered a sign of prior asbestos exposure, they have been found in patients exposed to other materials such as man-made vitreous fibers or certain silicates [10]. The prevalence of **asbestosis** in the United States is not known, there were an estimated 20,000 hospital discharges with a diagnosis of asbestosis in the year 2000, and approximately 2,000 deaths in which asbestosis was cited as the underlying or a contributing cause [9]. About 2000 deaths per year are thought to be due to **mesothelioma**, and an equal or slightly higher number of **lung cancers** claim asbestos as etiologic agent. A recent meta-analysis combining multiple cohorts concluded that asbestos kills at least twice as many people through lung cancer than through mesothelioma [11].

Mesothelioma is likely the most feared complication of asbestos exposure. All commercially valuable forms of asbestos, including amosite, crocidolite, and chrysotile along with its contaminant, tremolite, have been associated with mesotheliomas in humans and shown to produce mesotheliomas in experimental animals [12]. The health hazards associated with asbestos led to regulatory steps restricting the use of asbestos. While amosite usage ended around the mid 1970s, crocidolite was imported and remained in use until the mid 1990s [13, 14]. In 1989, the Environmental Protection Agency of the United States issued a final rule banning most asbestos-containing products. In 1991, this regulation was overturned by the an Appeals Court [15]. After
that, only a few asbestos-containing products remain banned. This ban includes so-called “new uses,” referring to the use of asbestos in products that historically have not contained asbestos.

### III. Role of Cytology in Asbestos-Related Disease

Cytopathology can be useful in the evaluation of patients with asbestos related diseases. The goal when encountering specimens from patients exposed to asbestos are (1) to diagnose the disease, and (2) to establish an etiologic relationship to asbestos exposure. It is probably fair to say that the former is much easier than the latter. Cytologic examination of exfoliative or aspiration biopsy is largely limited by its negative predictive value. Cytologic specimens usually represent very limited sampling of an assumed pathologic process, and an absence of pathologic findings not infrequently results in some degree of dissatisfaction. Studies have found no convincing evidence to support a role of routine sputum cytology in the early detection of bronchogenic carcinoma in asbestos workers [16]. It is assumed that readers are largely familiar with cytologic criteria for epithelial malignancies. Suffice it to say that any histologic type of lung carcinoma can occur in patients with exposure, and that the classification of epithelial malignancies in this patient population is the same as that used for those not exposed to asbestos [17, 18].

Most lung cancers today are diagnosed by bronchoscopic biopsies or image-guided fine needle aspirates or needle biopsies. Navigational systems have increased the diagnostic yield of bronchoscopic biopsies of small peripheral lesions [19]. Recent practice guidelines suggest obtaining sufficient tissue during the initial biopsy attempt
that allows for molecular testing of epidermal growth factor (EGFR) and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (K-RAS) mutations [17]. It is unclear whether K-RAS mutations, which are commonly seen in lung cancers of smokers, are more prevalent in those exposed to asbestos [20]. So far there is no association between EGFR mutations and asbestos-related lung cancers has been reported.

III.a Establishing an Diagnosis of Mesothelioma by Cytology

Mesothelioma is amenable to cytologic examination either by aspiration biopsy of the solid tumor or when the commonly associated effusion is drained. Mesothelioma cells normally do not exfoliate into sputum unless in those rare cases in which tumor extends into lung parenchyma [21]. One must be aware that the vast majority of malignant pleural effusions are secondary to adenocarcinoma. Only less than 1% are related to mesothelioma [22]. When evaluating a pleural effusion suspicious for malignancy, one must establish malignancy as well as determine the cell of origin.

Differentiating between metastatic adenocarcinoma and mesothelioma based on cytomorphic features alone is difficult. At low power, mesothelioma may be suggested by the presence of cell aggregates, consisting of “more and bigger cells” in “more and bigger clusters” [23-26]. Other features associated with mesothelial origin are peripheral cytoplasmic blebbing and formation of intercellular windows. Mesothelial cell clusters often have scalloped, or "knobby" borders, while clusters of adenocarcinoma are more frequently smooth and rounded. Papillary structures with fibrovascular cores can be seen in adenocarcinomas, in mesotheliomas and, albeit rarely, in benign pleural, pericardal and peritoneal effusions [27]. As a rule of thumb derived from surgical
specimens, the more pleomorphic the exfoliated cell population is, the more likely it represents adenocarcinoma and not mesothelioma. Mesotheliomas often show rather uniform populations of cells [27, 28].

**Immunohistochemical stains** are very useful to establish the lineage of an atypical cell population. A recently updated guideline addressing the details has been provided by the International Mesothelioma Interest Group [29]. The antibody panel used should be able to distinguish the entities in the differential diagnosis. One should be aware that (1) the various antibodies commonly employed have different specificities for mesothelial and adenocarcinoma cells, and (2) the majority of support for using these antibodies was generated using surgical and not cytology specimens. Antibodies commonly used to identify epithelioid mesothelial cells include calretinin, CK5/6, D2-40 (Podoplanin), WT-1 and HBME-1. Antigens used to identify carcinoma include CD15 (Leu M-1), Ber-EP4, B72.3 (TAG72), MOC-31, carcinoembryonic antigen (CEA), blood group 8 (BG8), estrogen receptor (ER), paired box proteins 2 and 8 (Pax-2, Pax-8), caudal type homeobox transcription factor 2 (CDX-2) and thyroid transcription factor 1 (TTF-1) [30-33]. Antibodies directed against ER, Pax-2, Pax-8 and CDX-2 are particularly useful to distinguishing peritoneal mesotheliomas from papillary serous carcinomas (ER), renal cell carcinomas (Pax-2, Pax-8) and intestinal carcinomas (CDX-2) [29]. The International Mesothelioma Panel recommends that at least two mesothelioma markers and two markers specific for the tumor in the differential diagnosis be used for a panel [29]. If these stains are conclusive, the diagnosis of mesothelioma may be considered established. In equivocal cases or suboptimal staining, a second, more expansive, round of immunohistochemical stains should be utilized.
Differentiating reactive from neoplastic mesothelial proliferations using immunohistochemical stains is much more difficult. Antibodies directed against insulin-like growth factor-II mRNA-binding protein 3 (IMP3), glucose transporter-1 (GLUT-1), E- and N-cadherin have shown some usefulness, but there is no uniformly “diagnostic” panel to achieve this task [34-37].

Detection of homozygous deletion of 9p21 by fluorescence in-situ hybridization (FISH) has been shown to be useful in distinguishing reactive from neoplastic mesothelial cells in effusion specimens [38]. Homozygous deletions are found in approximately two thirds of pleural mesotheliomas[ 39]. The 9p21 region harbors the p16 gene, a cyclin dependent kinase inhibitor, and may be more prone to damage by asbestos [40]. To date no reactive mesothelial proliferations have been reported to show this deletion, therefore, demonstrating this abnormality in a specimen appears to be specific for neoplasia. It is important to note that p16 deletions have been found in various other neoplasms, including lung, breast and urogenital cancers. The test should not be used to distinguish mesothelioma from adenocarcinoma. Another genetic abnormality found in about 25% of mesotheliomas are somatic mutations in the BAP1 gene, a tumor suppressor involved in BRCA1 regulation [41].

Electron microscopy may be able to help differentiate mesothelioma and adenocarcinoma. Most ultrastructural studies on this topic, performed on surgical and not cytology material, have advocated that long slender surface microvilli are characteristic of mesothelial cells. Sakuma et al. observed the same in exfoliated mesothelioma cells [42]. In addition, mesothelioma cells showed more abundant intermediate filaments and fewer free ribosomes, while reactive mesothelial cells contained fewer mitochondria.
In summary, making a diagnosis of mesothelioma on cytology material is fraught with some **diagnostic uncertainty**. It is up to the pathologist and his or her client how much diagnostic uncertainty they are willing to accept.

### III.b Establishing an Etiologic Role of Asbestos by Cytology

In order to establish the etiologic relationship with asbestos, one should try to find evidence of exposure in the form of asbestos bodies in fluid or tissue samples. Most importantly, there are no specific cytologic features to suggest or prove asbestos exposure. Asbestos bodies have not been identified in benign effusions.

**Sputum:** The number of asbestos bodies in sputa appears to be related to age and duration of exposure [43, 44]. Asbestos bodies appear in sputum when the asbestos burden is around 1000 asbestos bodies or more per gram of wet lung tissue [45, 46]. However, those exposed to asbestos may not exhibit asbestos bodies, and those without known exposure may show asbestos bodies [47]. Obviously this brings up the question whether patients who have asbestos bodies but no exposure history were exposed at some point in their life. Alderisio et al., for example, found no asbestos bodies in the sputa of 119 inhabitants of rural areas and only 1 asbestos body in a single sputum from a cohort of 164 traffic police officers [48]. The one affected officer was involved in inspecting illegal building construction. Finding asbestos bodies in sputum may not only be specific for exposure, it may also predict parenchymal lung disease [49].

**Bronchoalveolar lavage:** The presence of asbestos bodies in bronchoalveolar lavage (BAL) fluid also seems to be a marker for asbestos exposure. Finding more than one asbestos body per ml of lavage fluid is indicative of considerable exposure to
asbestos and associated with tissue levels of more than 1000 asbestos bodies per cm$^3$ of lung tissue [50, 51]. However, the false negative rate seems to be high. Putzu et al. found amphibole fibers in an Italian cohort by fiber analysis but no asbestos bodies by light microscopy [52]. Alexopoulos et al. found asbestos bodies only in 20% of chrysotile workers [53]. These studies highlight the problem that not all asbestos types show the same capacity to form ferruginous bodies. Some may thus escape detection by light microscopy in routine cytologic preparations.

**Fine needle aspiration:** Leiman found asbestos bodies in 52 (4%) of 1,256 thoracic aspiration biopsies of lung masses [54]. Forty-four (84%) of these patients had significant occupational asbestos exposure. About two thirds of the cases turned out to be malignant neoplasms, the others were benign lesions, typically abscesses or tuberculosis. The author concluded that the demonstration of asbestos bodies is highly associated with pulmonary pathology other than asbestosis. Still, the demonstration of asbestos bodies in aspiration biopsy specimens appears to be a marker of significant exposure. It also suggests that the aspirated lesion may be asbestos-related.

**IV. Making a Diagnosis of Mesothelioma on Cytology Material**

A common question is whether a definitive diagnosis of mesothelioma can be rendered using cytology material only. No matter how small the uncertainty of a mesothelioma diagnosis off cytology material may be, in light of the dramatic prognostic, therapeutic and medicolegal consequences of this diagnosis it is the presenter’s practice not to render a diagnosis of mesothelioma based solely on cytologic evaluation. Without ancillary immunohistochemical or electron microscopic studies, the post-test probability
of mesothelioma is very low, and making such diagnosis based only on light microscopic examination of cytologic material appears unwise. The post-test probability increased dramatically in situations in which immunohistochemical stains unequivocally support the mesothelial nature of the atypical cells. In these situations calculation of the post-test probability needs to consider atypical reactive as well as atypical neoplastic mesothelial cells. Even if the diagnostic probability under ideal circumstances approaches 90%, it is best left to the individual practitioner whether he or she feels comfortable rendering a diagnosis of mesothelioma rather than stating strong recommendations. The most promising test at this point is probably FISH testing for p16 deletion. If the mesothelial nature of the cells in questions is confirmed, homozygous deletion of 9p21 is highly suggestive of mesothelioma. The International Mesothelioma Interest Group has not rendered a recommendation to equate homozygous deletion of p16 with the presence of mesothelioma. Just as certain translocations are considered diagnostic of certain hematopoietic neoplasms in the proper setting, future experience and studies may permit similar diagnostic certainty for mesothelioma in the proper setting.

V. Reference


