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-K i-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 a 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 cytomorphologic 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, CK 5/6, D2-40 (Podoplanin), WT-1 and HBM E-1. Antigens used to identify carcinoma include CD15 (Leu M-1), Ber-EP4, B72.3 (TAG 72), 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 M esothelioma 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 cm³ 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 question 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

1. Rom, W.N., Asbestos related diseases, in Environmental and Occupational
2. Virta, R., Asbestos: Geology, Mineralogy, Mining, and Uses, U.S.G. Survey,
5. Morgan, A. and A. Holmes, The enigmatic asbestos body: Its formation and


