International Early Lung Cancer Action Program: Pathology Protocol

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In research on CT-based screening for lung cancer, the Early Lung Cancer Action Project (ELCAP) (1, 2) has been followed by New York ELCAP (NY-ELCAP) and International ELCAP (I-ELCAP). In all of these the protocol is in principle the same, except that the original ELCAP protocol has recently been updated in various particulars other than pathology (3). The original ELCAP and the NY-ELCAP were conceived as projects, limited in their time horizons, while the I-ELCAP is a program of ongoing international collaboration in screening research on lung cancer.

Central to the success of these efforts is assurance of high-quality early *diagnosis* of lung cancer. CT-based detection of a nodule is the beginning, and CT-based assessment of the nodule's rate of growth commonly the next step; but conclusive detection of lung cancer always rests on *pathologic* facts and their interpretation.

The pathology protocol presented here is principally directed to quality-assurance for the diagnosis of presence/absence of malignancy (4), but there is a larger purpose as well: the recent International Conferences on Screening for Lung Cancer (5) have emphasized the importance of pathologic characterization of the diagnosed early cancers also for the purposes of further refining of diagnostic criteria as well as staging and other prognostic criteria, and for more basic research as well.

This protocol addresses cytologic and histologic aspects of the research pathology as separate topics; and under each of these topics, it addresses the research role of the pathologists in the participating institutions ('sites') for one and the central reading and interpretation for another. In no way does this protocol represent guidelines for diagnostic practices in the participating institutions, nor does the central, research-oriented diagnosis represent second opinion for patient care.

I. Cytology

The cytologic specimen is a fine-needle aspirate obtained from a CT-detected abnormality either by percutaneous or bronchoscopic biopsy procedure. If more than one CT abnormality has been aspirated, each specimen is labeled as to its location.

The site cytologist smears part of the specimen as diff-quick immediately on receiving the aspirate, and the remainder is preserved in alcohol for Papanicolaou staining. The needles are rinsed in Cyto-Lyt solution, as needed, and the spin-down is put on glass slides for staining. The blood clot is fixed in formalin and sent for histologic examination as a cell block.

For study purposes, the routine diagnostic report and the entire set of slides pertaining to the case are sent to the Coordinating Center, the latter for the Expert Cytology Panel to examine and document.

The set of slides for each CT abnormality is read independently of the site diagnosis by one member of the Expert Cytology Panel, and the descriptive findings are recorded on the ELCAP Cytology Form according to stated categories (see Appendix I). The 'solo' expert also translates

the findings to diagnosis according to the World Health Organization categories (Appendix II) (6). The other panel members independently review the set of slides for the case and record their diagnoses. When there is a discrepancy among the members, a consensus diagnosis is produced. A record is kept of the diagnoses at the site, by the solo expert and each of the other expert panel members, as well as of the consensus diagnosis. All slides are be copied into a digital file before returning them to the site. At least one representative slide of each CT abnormality will be kept at the Coordinating Center for tissue banking purposes.

As the slides on the case are examined by the solo expert reviewer, the following findings are documented as input facts for the diagnosis, each on a five-point Likert scale: cell size (small to large), nuclear grade (low to high), isonucleosis (isonucleosis to anisonucleosis), chromatin pattern (fine to coarse), nucleoli (absent to prominent) mitotic activity (low to high), nuclear hyperchromasia (absent to prominent), nuclear/cytoplasm ratio (normal to increased), nuclear membrane (smooth to irregular) cell borders (distinct to indistinct), monomorphic/polymorphic (monomorphic to polymorphic), nuclear crowding (absent to marked), polarity (present to abundant), cellularity (spare to abundant), cohension/dyshesion (cohesive to dyshesive), background necrosis (absent to abundant), extracellular mucin (absent to abundant), histocytic infiltrate (absent to abundant), histocytic infiltrate (absent to abundant), giant cell infiltrate (absent to abundant), inflammation severity (insignificant to severe), intranuclear inclusions (absent to abundant), intranuclear cytoplasmic invaginations (absent to abundant), intracellular mucin (absent to abundant).

II. Gross pathology and histology

Gross specimens derive from thoracotomy or thoracoscopy, core specimens from CT- or bronchoscopy-guided biopsy procedures.

The site pathologist immediately fixes the core biopsy specimen in formalin for four hours. Then, 4-micron (or 6-micron, if routine) sections are cut for histologic examination.

The site pathologist measures the wedge specimen in three dimensions, removes the staples and fixes it in formalin for at least 24 hours, with the container with the specimen shaken to prevent specimen atelectasis. The specimen is then cut into 5-mm sections for gross inspection and tissue blocks are cut at 4-micro (or 6-micron if routine) for histologic examination.

The site pathologist also measures the lobectomy/pneumonectomy specimen in three dimensions, inflates it with formalin through the main bronchus or bronchi, and allows specimen fixation for at least 24 hours. Trans-axial sections are then cut at 5-mm intervals.

The site pathologist documents the gross findings on the specimen. Each lesion is described as to location, size in three dimensions, shape, consistency, necrosis, relation to pleura, airways, bronchial and stapled resection margins. Normal lung is described as to color, consistency, enlargement of airspaces, airway lesions and peritumoral changes. The cartilage-bearing airways are described as to diameter, mucosal color (pink, red, dark red), consistency (soft, firm) and intraluminal material or lesions (mucus, blood clot). Lymph nodes

are described as to location (levels 1-12), size in three dimensions, color (pink, red, black), and consistency (soft, firm). The pleura is described as to color (pink, red, with or without black pigments), finish (shiny, dull), thickness (in cm), presence/absence of adhesions and lesions.

After completion of the gross specimen examination, the entire tumor or *at least ten* sections of tumor, all additional lesions, the bronchovascular resection margin, all peribronchial/hilar lymph nodes as well as *at least ten sections* of non-neoplastic lung should be "cassetted" and processed in a routine manner resulting in paraffin-embedded tissue and hematoxylin and eosin or hematoxylin phloxin and saffron-stained 4-micron (6-micro, if routine) tissue sections suitable for microscopic examination.

The gross description is narrated and submitted to the Coordinating Center together with the entire set of slides pertaining to the case, for examination and documentation by the Expert Pathology Panel.

The set of slides for each case is read independently of the site diagnosis by the Coordinating Center pathologist and a 'solo' member of the Expert Pathology Panel and the descriptive findings are recorded on the ELCAP Histology Form (see Appendix I). The expert also translates the findings to diagnosis according to the World Health Organization categories (Appendix II) (5). The other panel members independently review the set of slides for the case and record their diagnoses. When there is a discrepancy among the expert diagnoses or about the descriptive findings, a consensus diagnosis/finding is produced. A record is kept of the diagnosis/findings at the site, by the Center pathologist and 'solo' expert and each of the other members, as well as of the consensus diagnosis. All slides are be copied into a digital file and a limited set of slides of the tumor (one representative slide from each block) and of normal tissue (one representative slide from each block) will be kept at the Coordinating Center for tissue banking purposes.

As the slides on the case are examined by the Center pathologist and solo expert reviewer, the following findings are reported as input facts for the diagnosis: size in three dimensions, cell type, degree of differentiation, nuclear grade, fibrosis, necrosis, granulomatous response, presence/ absence of each of: stromal invasion, pleural invasion, angiolymphatic invasion, bronchial invasion, satellite lesions (with complete descriptions of these lesions) and precursor lesions (atypical adenomatous hyperplasia, squamous metaplasia and/or dysplasia and pneumocye proliferations of uncertain malignant potential). The acellular mucinous component of mucinous tumors and cystic component of partially cystic tumors are included in size measurements. Tumor disruption by previous diagnostic procedures (i.e., FNA needle tract(s)) are also be noted. The non-neoplastic lung is carefully studied for the presence of satellite tumors (site, size, type) and all other findings including evidence of previous diagnostic procedures, i.e., fine needle aspirate needle tract, tobacco-related diseases such as emphysema and respiratory bronchiolitis are noted. Pre-invasive lesions (squamous cell dysplasia, atypical adenomyatous hyperplasia, and neuroendocrine) are similarly documented. The consensus WHO tumor category is recorded.

References

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2. Henschke CI, Naidich DP Yankelevitz DF, McGuinness G, McCauley DI, Smith JP, Libby DM, Pasmantier MW, Koizumi J, Vazquez M, Flieder D, Altorki NK, Miettinen OS. Early Lung Cancer Action Project: Initial findings from annual repeat screening. Cancer. 2001, July 1.

3. Henschke CI, Yankelevitz DF, Smith JP, Miettinen OS for the ELCAP Group. International early lung cancer action program: Origins, aims, principles and protocol. Lung Cancer. 2002; 35: 143-148

4. Vazquez M, Flieder D, Travis W, Carter D, Yankelevitz D, Miettinen OSM, Henschke CI. Early Lung Cancer Action Project Pathology Protocol. Lung Cancer 2003;39:231-32

5. International Conferences on Screening for Lung Cancer. www.IELCAP.org International Early Lung Cancer Action Program Conferences and Consensus Statements. www.IELCAP.org

6. World Health Organization: The World Health Organization Histological Typing of Lung Tumors, ed. 3, Geneva, Switzerland 1999.

APPENDIX I – CTYOLOGY DIAGNOSTIC CATEGORIES

The major diagnostic categories are given below. Further differentiation into subcategories can be recorded in the Cytologic Form:

Normal:

Atypical typical bronchioloalveolar proliferation:

Squamous cell carcinoma: a tumor in which the cells resemble stratified squamous epithelium

Adenocarcinoma: a tumor in which the neoplastic epithelial cells grow in a glandular pattern

Small cell carcinoma: a tumor in which the neoplastic cells are derived from neuroendorcine cells of the lung. The tumor cells are small, dark, round-to-oval, lymphocyte-like cells that have scant cytoplasm and hyperchromatic nuclei.

Large cell carcinoma: a group of tumors that lack cytologic differentiation and probably represent squamous cell and glandular neoplasms that are too undifferentiated to permit categorization

Adenosquamous carcinoma: a tumor with combined squamous and adenocarcinoma pattern

Carcinoma with pleomorphic sarcomatoid/sarcomatous elements: a poorly differentiated carcinoma with spindle cell components

Carcinoid tumor: a tumor in which neoplastic cells show the neuroendocrine differentiation of Kulchitsky cells in the bronchial mucosa and resemble intestinal carcinoids. The cells contain dense core neurosecretory granules in their cytoplasm and rarely may secrete hormonally active polypeptides. This tumor should be further classified as typical or atypical.

Carcinomas of salivary gland type: a group of tumors whose cells derive from the salivary gland

APPENDIX II - WHO HISTOLOGY DIAGNOSTIC CATEGORIES

Abstracted from the World Health Organization Histological
Typing of Lung Tumors, ed. 3, Geneva, Switzerland 1999.

1. Normal
2. Atypical bronchioloalveolar proliferation:
3. Squamous cell carcinoma: a tumor in which the cells resemble stratified squamous epithelium
    Variants: papillary, clear-cell, small-cell, basaloid
4. Small cell carcinoma: a tumor in which the neoplastic cells are derived from neuroendorcine cells of the lung. The tumor cells are small, dark, round-to-oval, lymphocyte-like cells that have scant cytoplasm and hyperchromatic nuclei.
    Variants: combined small-cell carcinoma
5. Adenocarcinoma: a tumor in which the neoplastic epithelial cells grow in a glandular pattern
    Variants: acinar, papillary, bronchioloalveolar carcinoma
        Variants of bronchioloalveolar carcinoma: non-mucinous (Clara cell/type II pneumocyte type, mucinous (Goblet cell type), mixed mucinous and non-mucinous (Clara cell/type II pneumocyte and goblet cell type), indeterminate
6. Solid adenocarcinoma with mucin formation
7. Adenocarcinoma with mixed subtypes
    Variants: well-differentiated fetal adenocarcinoma, mucinous ('colloid") adenocarcinoma, mucinous cystadenocarcinoma, signet ring adenocarcinoma, clear cell adenocarcinoma
8. Large cell carcinoma: a group of tumors that lack cytologic differentiation and probably represent squamous cell and glandular neoplasms that are poorly undifferentiated to permit categorization
    Variants: large cell neurendocrine carcinoma, basaloid carcinoma, lymphepithelioma-like carcinoma, clear cell carcinoma, large cell carcinoma with rhadoid phenotype
9. Adenosquamous carcinoma: a tumor with combined squamous and adenocarcinoma patterns
10. Carcinoma with pleomorphic sarcomatoid/sarcomatous elements: a poorly differentiated carcinoma with spindle cell components
    Carcinoma with pleomorphic, sarcomatoid or sarcomatous elements
    Carcinoma with spindle and/or giant cells
        Variants: pleumorphic, spindle cell, giant cell
    Carcinosarcoma
    Pulmonary Blastoma
    Other
11. Carcinoid tumor: a tumor in which neoplastic cells show the neuroendocrine differentiation of Kulchitsky cells in the bronchial mucosa and resemble intestinal carcinoids. The cells contain dense core neurosecretory granules in their cytoplasm and rarely may secrete hormonally active polypeptides.
    Variants: typical or atypical.

12.Carcinomas of salivary gland type: a group of tumors whose cells derive from the salivary gland

Variants: mucoepidermoid carcinoma, adenoid cystic carcinoma, other

13.Unclassified carcinoma

14.Pleural involvement: P0, P1, P2, P3

P0: tumor with no pleural involvement or reaching the visceral pleura but not extending beyond its elastic pleural layer
P1: tumor reaching visceral inner pleura but not exposed on the pleural surface
P2: tumor exposed on the pleural surface
P3: tumor invading parietal pleural or chest wall.

Pleural invasion is present if P2 or P3.