INTRODUCTION TO PATHOLOGICAL TECHNIQUES

1. Types of biopsy procedures
2. Special exams
Biopsy-Indications

• Diffuse/multifocal lesions
  – Etiology of the disease
  – Evaluation of tumor characteristics for systemic treatment planning

• Solitary lesions
  – Etiology, dignity assessment
  – Evaluation before surgery
Biopsy types

• Cytology sampling
  – Exfoliative (brush)
  – Liquid
  – Fine needle aspiration

• Tissue sampling
  – By excision (direct, open surgical, video-assisted)
  – Core needle biopsy
  – By endoscopy
Biopsy-Guidance

• Visual
  – Superficial localization, body cavities, hollow organs

• By imaging (US, CT, MRI)
  – Deep localization
Cytology sampling

• Result: SMEAR= cell samples spread on a glass slide
  – Cellular elements: from the lesion and surrounding tissue (their ratio depends on sampling technique, type of lesion)
  – „Background”: blood, inflammatory cells, extracellular substance (mucus, colloid etc)

• Fast results (bedside diagnosis)

• Sample processing:
  – Wet fixation(alcohol)+staining (HE, Papanicolaou): preserved cellular morphology
  – Air drying+staining (Giemsa, Diff-Quik): fast and simple but alters cellular morphology
Cytology sampling - types

Exfoliative cytology (brush)

- Superficial lesions of hollow organs = intraepitelial or invasive tumors (cervix, small bronchus, biliary duct system)
- Sample characteristics: numerous normal/reactive epithelial cells
- Limitations
  - Reactive or malignant?
  - Dysplasia or invasive tumor?
Cytology sampling- types

Cytology of Liquids

• Body cavity effusions of neoplastic or inflammatory origin, cyst content, other fluids than blood (e.g. peritoneal, pleural, pericardial, urine)

• Sample characteristics
  – Numerous normal/reactive mesothelial or epithelial cells altered by liquid environment
  – Numerous inflammatory cells (neutrophils, histiocytes)

• Limitations
  – Reactive or malignant?
Cytology sampling- types

Fine needle aspiration (FNA)
• Solitary/multifocal solid lesions
• Sample characteristics
  – Tumor cells mainly (in case of a neoplastic process)
  – Surrounding tissue cellular elements in varying proportion (e.g. lymphoid cells if sample taken from a lymph node)
  – Contamination from needle track (e.g. if biopsying an abdominal mass: intestinal epithelial cells, mesothelial cells may also be present)
• Limitations
  – Sample not representative (missed targeting, necrosis, etc.)
Fine needle aspiration (FNA)
• Simple tools (needle, syringe)
• Guidance
  – US (first choice method, simple, fast, real time image)
  – EUS (lesion close to a hollow organ e.g. pancreas, hilar lymph nodes)
  – CT scan (lesion non-detectable by US, thoracic lesions, long procedure, targeting based on a still image)
Tissue sampling

• Result: SLIDE

• Time consuming (min. 24 hours-2 days)

• Formalin fixation
  – EXCEPT:
    • fresh sample from skin or kidney sent to pathology without delay! (immunofluorescent microscopy)
    • lymphomas (ideally fresh frozen sample for molecular techniques)
Tissue sampling types

Biopsy by endoscopy

- Gastroscopy (esophagus-duodenum)
- Colonoscopy (terminal ileum-anus)
- Laryngoscopy (pharynx-larynx)
- Bronchoscopy (trachea-large bronchi)
- Cystoscopy

- Focal lesions (tumor): 2-3 representative samples, from the periphery or surface of the lesion, not from necrosis!
- Diffuse lesions (gastritis, IBD): map biopsy
  - Ideal biopsy: representative= includes muscularis mucosae also, fixation on a flat surface=better orientation of the specimen while processing...
Tissue sampling types

Core needle biopsy

• Focal lesion (solitary or multifocal), solid organs – may be alternative/ancillary to cytology

• Diffuse lesions in solid parenchymal organs leading to structural alterations (e.g. glomerular diseases, diffuse hepatic lesions)

• Targeting: US, CT, MRI, stereotaxic
A core needle biopsy allows more tissue to be removed from the breast. This allows the pathologists to give a histological diagnosis as against a cytological diagnosis obtained by FNA.
# Cytology vs tissue sampling

<table>
<thead>
<tr>
<th></th>
<th>Cytology</th>
<th>Histology</th>
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<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td>• fast</td>
<td>• Several slides from the same sample</td>
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<td></td>
<td>• Simple tools</td>
<td>• Ideal if immunohistochemistry evaluation is needed</td>
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<td>• Minimally invasive, complications rare</td>
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<td></td>
<td>• Several slides from the same sample</td>
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<tr>
<td><strong>Disadvantages/limitations</strong></td>
<td>• Limited sample(smear)</td>
<td>• Time consuming processing</td>
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<td>• Ancillary exams (e.g. immunohistochemistry) limited</td>
<td>• More expensive, lab requirements</td>
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<td></td>
<td></td>
<td>• Invasive, complications may occur</td>
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<tr>
<td><strong>Diagnostic evaluation(tumors)</strong></td>
<td>• Dignity</td>
<td>• Dignity</td>
</tr>
<tr>
<td></td>
<td>• Type – main tumor type</td>
<td>• Type – more accurate tumor typing</td>
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<tr>
<td></td>
<td>• Low grade/high grade</td>
<td>• Grade-assessment of proliferation</td>
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<tr>
<td></td>
<td>• Invasion – limited</td>
<td>• Invasion</td>
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<td><strong>Setting</strong></td>
<td>• Before surgery</td>
<td>• Before surgery</td>
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<td>• in case of a metastatic disease clarify etiology</td>
<td>• Systemic therapy planning</td>
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<td>• Some special tumors (e.g. lymphomas)</td>
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Both techniques require experience!!!! Unsatisfactory samples are not diagnostic-unnecessary invasive intervention!
Intraoperative exam

Indications

• No preoperative biopsy (e.g. pancreas, ovarium): to evaluate dignity (benign or malignant)

• In case of a known malignancy:
  – Resection margin assessment (positive or negative)
  – Sentinel lymph node biopsy (positive or negative)
  – Unrecognized lesion by preoperative imaging (e.g. liver metastasis or carcinosis)
Intraoperative exam

Technics

- Intraoperative cytology (FNA): by the surgeon (on palpation, US-guided)
- Intraoperative tissue sampling: quick-frozen section (cryostat), H&E staining (10-20 minutes) – morphology altered by low temperature, structure mainly preserved (invasion?, resection margins?)
- Touch prep: ancillary to frozen section: cellular morphology preserved (e.g. evaluating tumor cell nuclei)
Special exams

- Protein-based techniques: immunohistochemistry, immunocytochemistry
- Molecular pathology: DNA/RNA-based exams
  - FISH (morphology-based..)
  - Sequence analysis etc. (see lectures)
Immunohistochemical reaction

Definition

Detection of proteins or protein fragments by immunological reaction (antigen-antibody complex). Generally used in tumor pathology

• Normal proteins which show the cellular origin of a tumor

• Abnormal accumulation of proteins during a pathological process (malignant transformation)
# Diagnostic markers

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Marker(s)</th>
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<tr>
<td>Epithelial tumors (carcinoma)</td>
<td>Cytokeratin subtypes, tissue-specific markers (PSA, TTF-1, etc.)</td>
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<tr>
<td>Mesenchymal tumors</td>
<td>Tissue specific markers (actin, s-100, factor VIII, etc.)</td>
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<td>Hematologic tumors</td>
<td>CD proteins (T/B cell markers, etc.)</td>
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<tr>
<td>Undifferentiated tumors</td>
<td>CK, vimentin, Melan-A, CD45 = LCA</td>
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# Prognostic/predictive markers

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<tr>
<th>Prognosis</th>
<th>Proliferation: Ki-67 Oncoprotein mutation, accumulation: p-53</th>
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<td>Predictive markers (to targeted therapies)</td>
<td>Hormon receptors: ER Growth factor receptors: EGFR, HER2, c-KIT</td>
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Commonly used IH reactions

• Normal proteins
  – Cytoskeleton (*cytoplasmic reaction*): cytokeratin (*epithelium*), vimentin (*mesenchymal cell*), S-100 (*neuron*), actin (*muscle*) etc..
  – Receptor (*membrane or nuclear reaction*): estrogen receptor, progesteron receptor (breast), CD proteins (hemato-lymphogen cells)
  – Cell cycle regulators (*nuclear reaction*): MIB-1/Ki-67
  – Other (cellular adhesions, cytoplasmic compartment, enzymes etc..)

• Abnormal protein accumulation
  – Oncoproteins (p-53, growth factor receptors: EGFR, HER2)
  – Infective agents (viral compartments)
  – Other (tau proteins in neurodegenerative diseases)
Method of immunohistochemistry

- Primary antibody (antigen specific)
- Secondary antibody+chromogen (visual detection)
FISH (fluorescent in situ hybridisation)

- Detecting specific DNA sequences within chromosomes
- Tumor pathology
  - Amplification, deletion, translocation detections
  - Predictive and diagnostic exams
- Microbiology
  - Species specific