# INTRODUCTION TO PATHOLOGICAL TECHNIQUES

1. Types of biopsy procedures
2. Special exams

# Biopsy-Indications

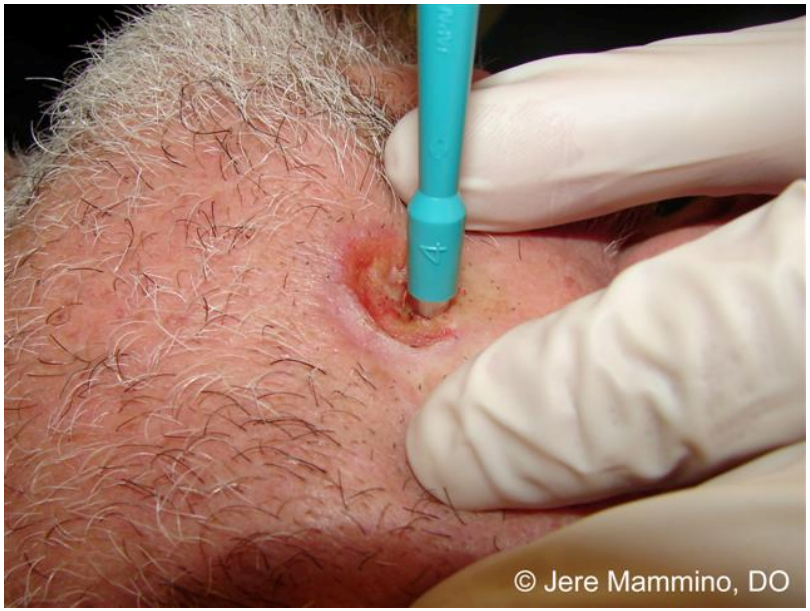
- Diffuse/multifocal lesions
  - Etiology of the disease
  - Evaluation of tumor characteristics for systemic treatment planning
- Solitary lesions
  - Etiology, dignity assessment
  - Evaluation before surgery

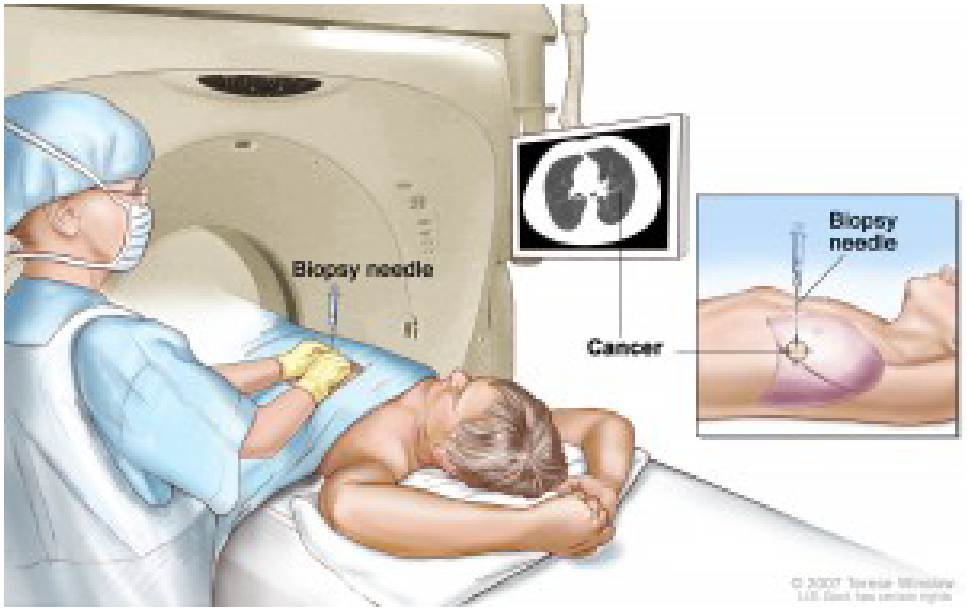
# Biopsy types

- 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



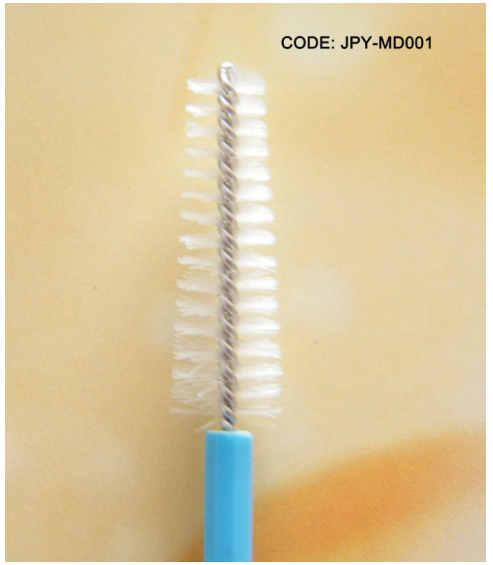
# Cytology sampling

- 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?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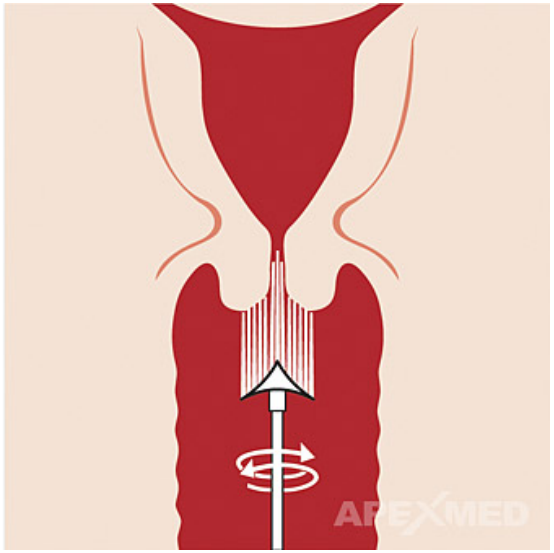
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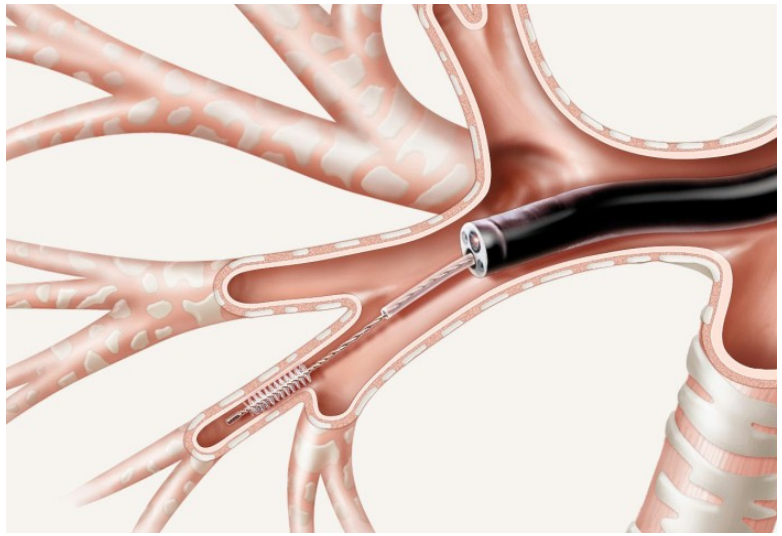


# Cytology sampling- types

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?

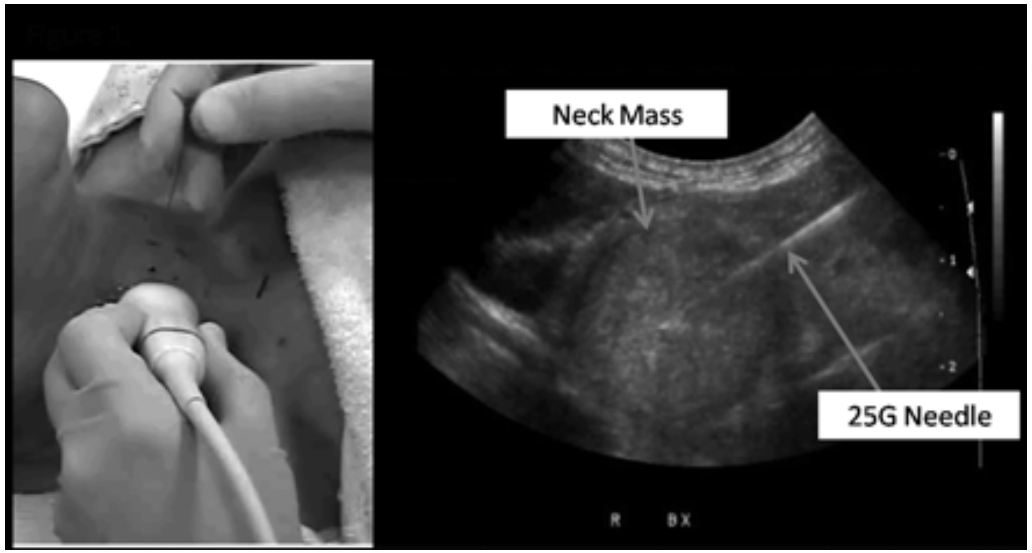
# Cytology sampling- types

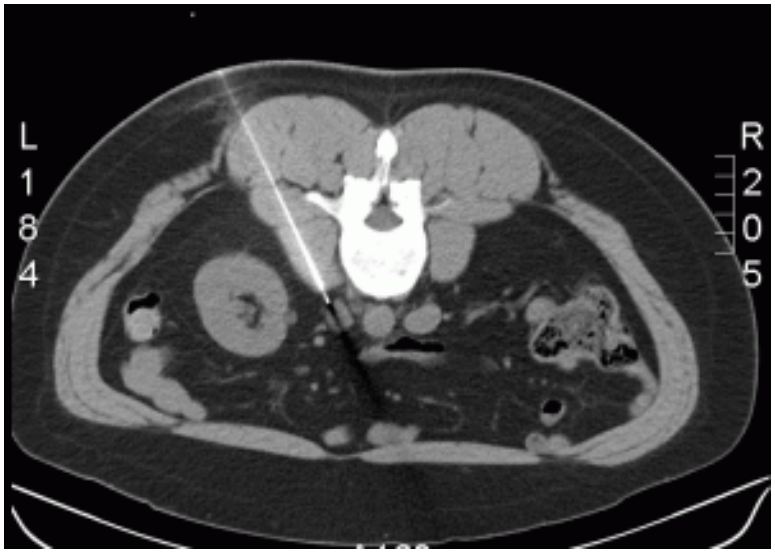
Fine needle aspiration (FNA)

- 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

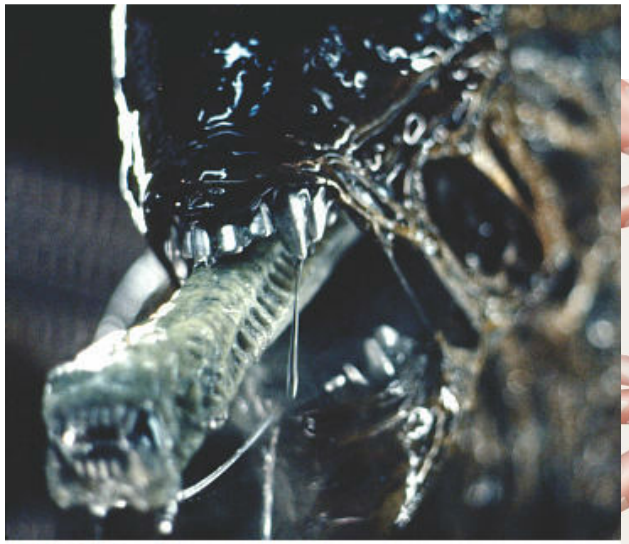
- Result: SLIDE
- Time consuming (min. 24 hours-2 days)
- Formalin fixation
  - EXCEPT:
    - fresh sample from skin or kidney sent to pathology without delay! (immunofluorescent microscopy)
    - lymphomas (ideally fresh frozen sample for molecular techniques)

# Tissue sampling types

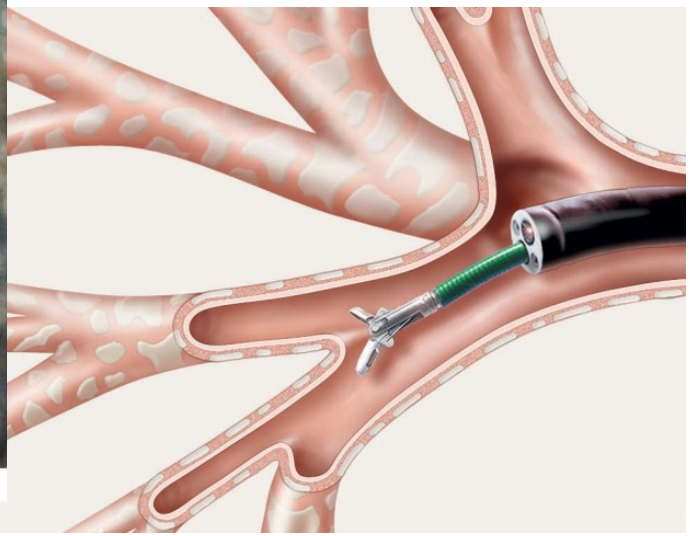
Biopsy by endoscopy

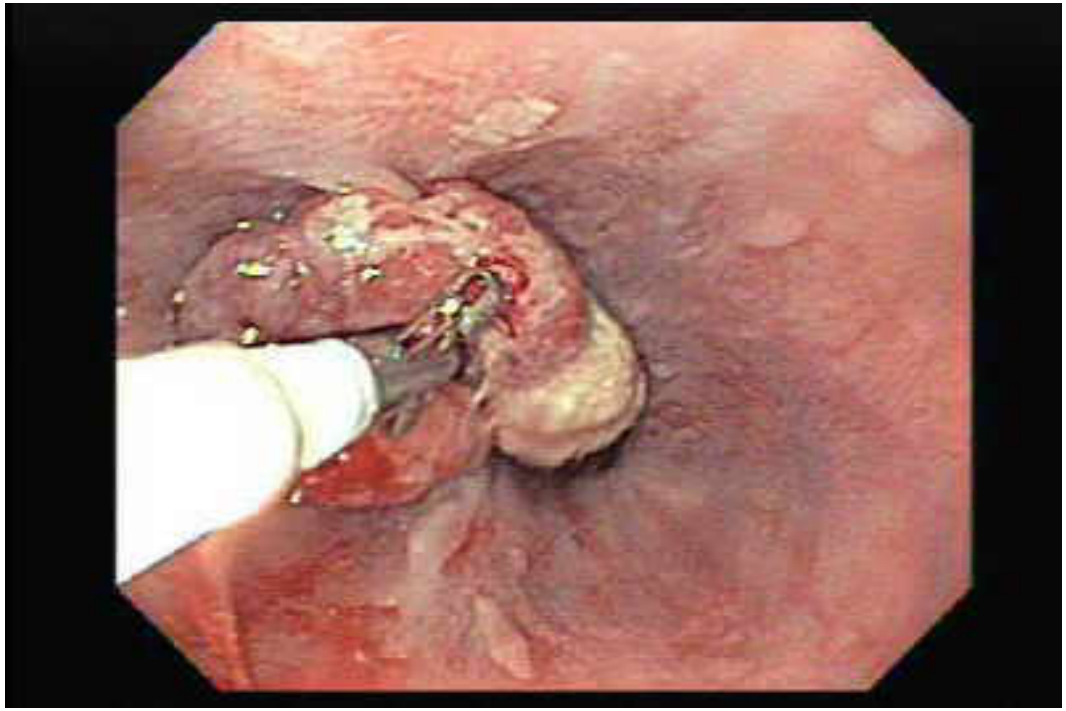
- Gastroscopy (esophagus-duodenum)
- Colonoscopy (terminal ileum-anus)
- Laryngoscopy (pharynx-larynx)
- Bronchoscopy (trachea-large bronchi)
- Cystoscopy

- 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…

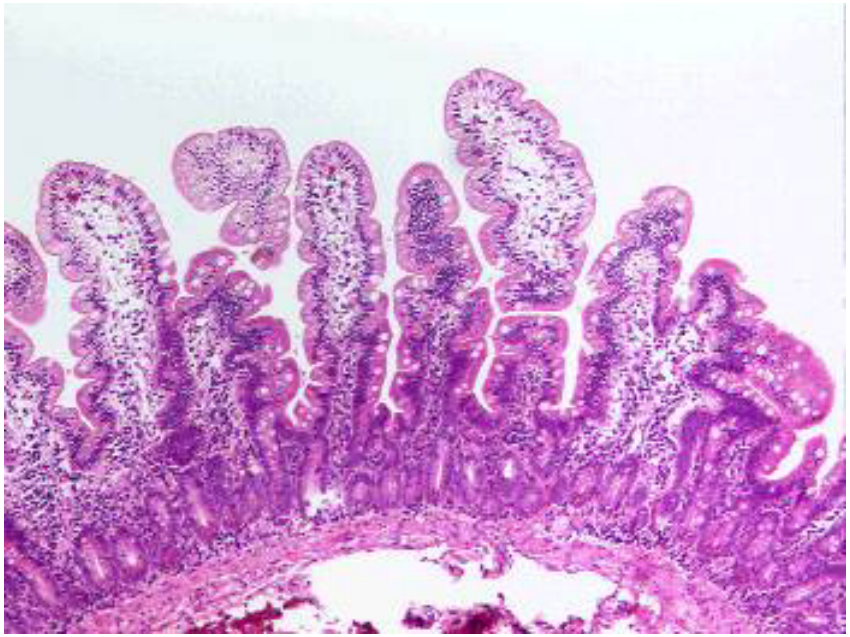


20th Century Fox

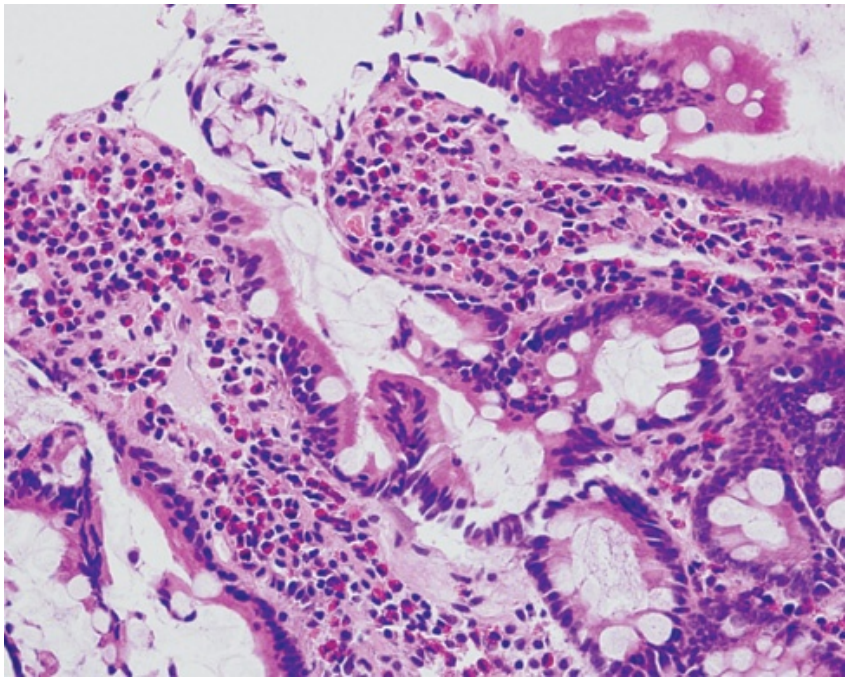




Ideal…



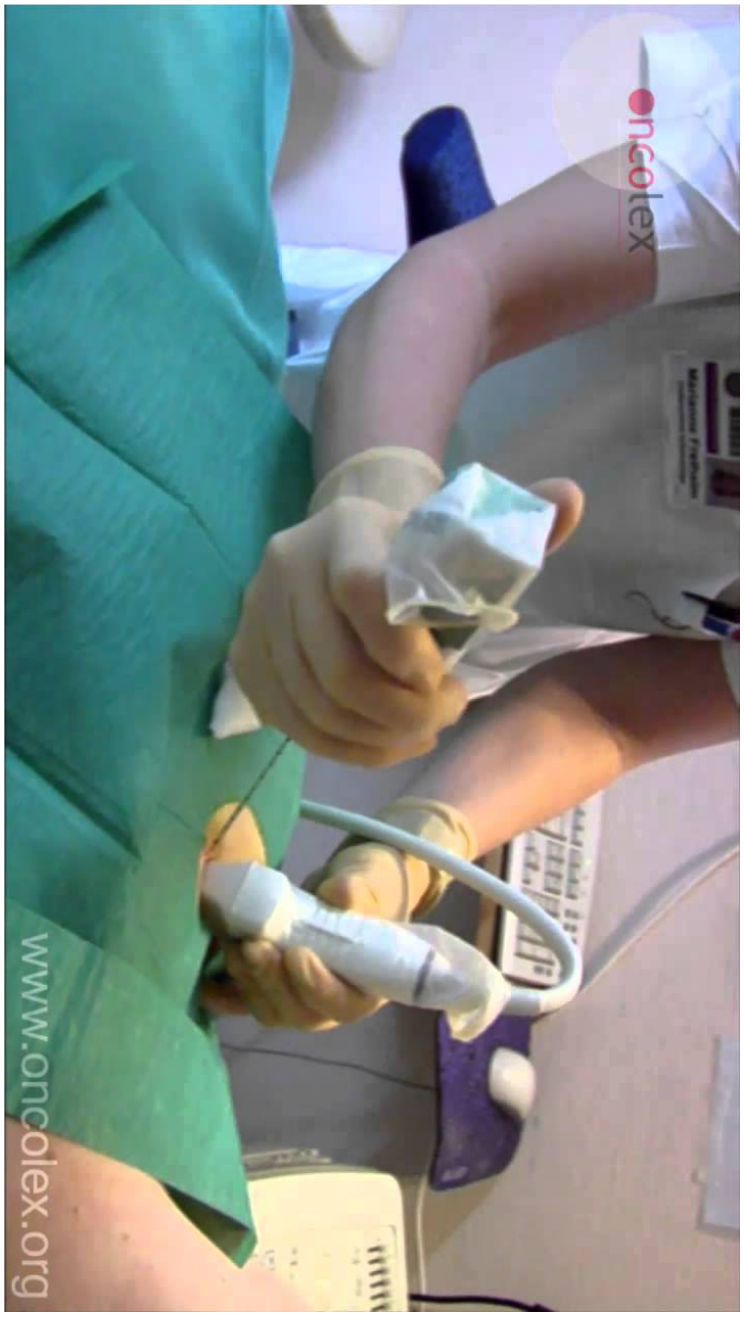
Suboptimal…

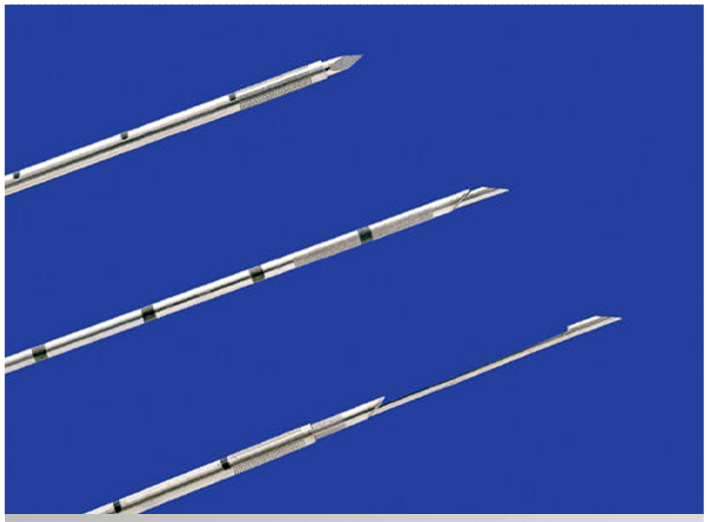


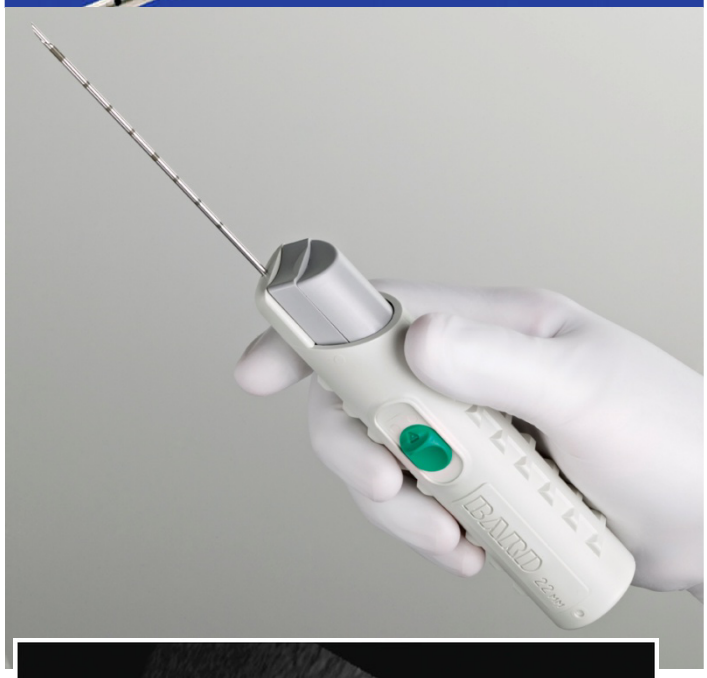
# Tissue sampling types

Core needle biopsy

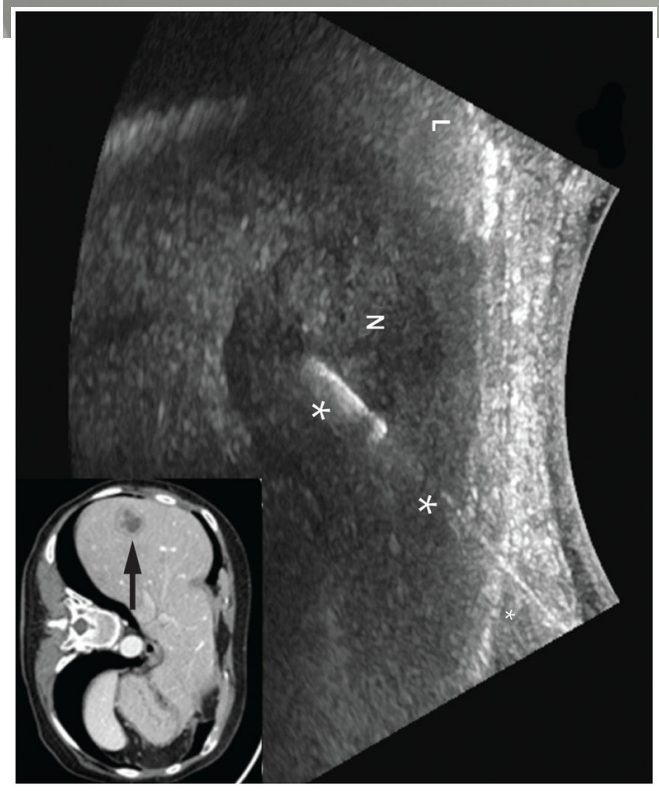
- Focal lesion (solitary or multifocal), solid organs – may be  alternative/ancillary to cytology
- Diffuse lesions in solid parenchymal organs leading to structural alterations ( e.g. glomerular diseases, diffuse hepatic lesions)
- Targeting: US, CT, MRI, stereotaxic






A core needle biopsy allows more tissue to be removed from the breast. This allows the pathologist to give a histological diagnosis as against a cytological diagnosis obtained by FNAC



# Cytology vs tissue sampling

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

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

# Prognostic/predictive markers

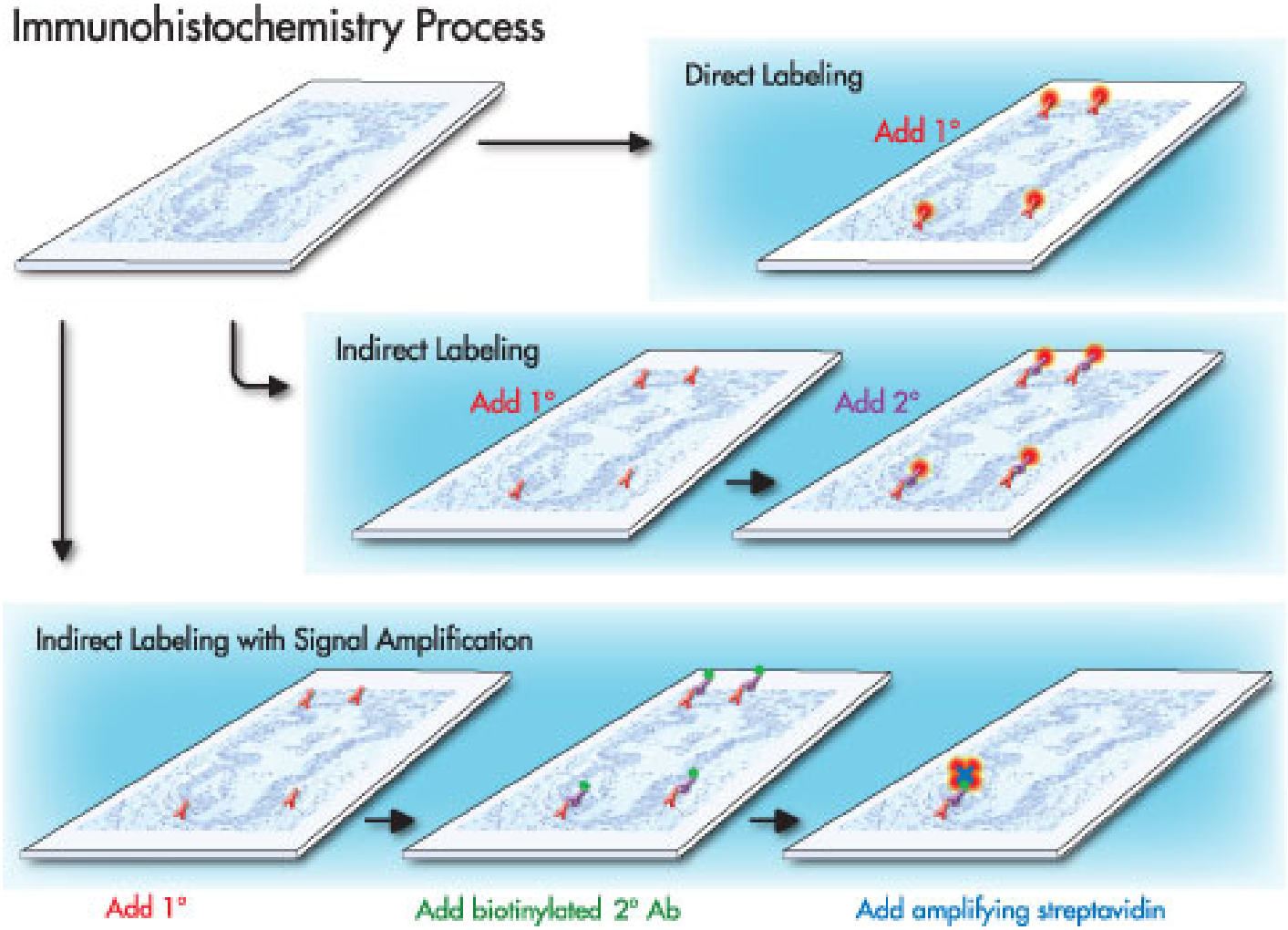
| | |
|---|---|
| **Prognosis** | Proliferation: Ki-67<br>Oncoprotein mutation, accumulation: p-53 |
| **Predictive markers (to targeted therapies)** | Hormon receptors: ER<br>Growth factor receptors: EGFR, HER2, c-KIT |

# 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 reactio*n): 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 patology
  - Amplification, deletion, translocation detections
  - Predictive and diagnostic exams
- Microbiology
  - Species specific