TUMOR MARKERS

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• substances which can be related to the presence or progress of a malignancy

• antigens expressed on the tumor cells surfaces - *cellular tumor markers*

• different substances (found in the blood or other body fluids) produced by the tumor cells (*Tu-associated m.*) or by an organism (*induced m.*) in the presence of tumor - *humoral tumor markers*

• **The ideal marker should be both specific for a given type and localization of cancer and sensitive enough to detect small tumors for early diagnosis or during screening.**
UTILIZATION OF TUMOR MARKERS

- **Screening**: *calcitonin* in families with MEN syndrome, *AFP* in patients with liver cirrhosis, *PSA* in men >50 years
- **Dg and diff. dg** in symptomatic individuals
- **Clinical staging of cancer**, is aided by quantitation of the marker, i.e. the serum level of the marker reflects the number of cancer cells present in the body
- **Monitoring** of the disease and estimation of tumor value
- **Prognostic indicator** of disease progression and patient survival
- **Detection of cancer recurrence**, permits early treatment or a change in therapy
- **Monitoring of responses to therapy**
CLASSIFICATION OF TUMOR MARKERS

• According to chemical structure

• According to visceral specificity

• According to function
Chemical structure

- glykoproteins
- glykolipids
- peptides
- imunoglobulins
- polyamines
- carbohydrates
Visceral specificity

- **high:** calcitonin - medullary carcinoma of the thyroid
  PSA - prostate cancer
  NSE - small cell lung cancer
  hCG - germ-cell tumors
  AFP - hepatocellular and germ-cell carcinoma

- **moderate:** CA 19-9 - pancreatic cancer
  CA 125 - ovarian cancer
  CA 15-3 - breast cancer

- **low:** CEA
  TPA
Function

- oncofetal antigens
- oncoplacental antigens
- enzymes
- hormones
- serum proteins
- receptors
- others
Oncofetal antigens

• substances produced during fetal life
• present in high concentrations in the sera of fetuses, decrease to low levels or disappear after the birth
• reappear in patients with cancer
• Their production demonstrates that certain genes are reactivated as a result of the malignant transformation of the cell.

• examples: AFP, CEA, CAs, CYFRA 21-1, SCC, MCA, MSA, TATI
Oncoplacental antigens

- produced by the trophoblastic cells of the placenta in both pregnancy and pathological conditions and also by germinative tumors as a mark of malignant dedifferentiation
- ↑ levels show evidence of ↑ malignancy and metastatic potency of the given tumor

- examples: *hCG, SP-1*
Hormones

• The production of hormones in cancer involves two separate routes:
  1. the endocrine tissue that normally produces the given hormone can produce its excess amounts
  2. ectopic syndrome - hormone produced by a distant nonendocrine tissue that normally does not produce this hormone

• for instance: ACTH normally produced by the pituitary gland ectopically produced by the lung small cells
• elevation of a hormone is not specific ← it may be produced by a variety of cancers

• examples: ACTH, ADH, PTH, calcitonin, STH, prolactin
Enzymes

• present in much higher concentrations inside cells
• released into circulation as the result of tumor necrosis or a change in the membrane permeability of the cancer cells →
• elevated enzyme levels may signal the presence of malignancy but usually are not specific enough to identify a cancer type or organ involvement

• examples: *NSE, TK, ALP, PSA, LD*
Serum proteins

• produced either by tumor cells or by an organism in the presence of tumor

• non-specific

• monitoring

• examples: $\beta_2$-microglobulin, ferritin, paraprotein
Receptors

- cellular markers used in hormone-producing tumors

- examples: *estrogen* and *progesterone receptors*
Other tumor markers

• tissues - produced substances, which we cannot class with the previously mentioned groups

• examples: **TPA, TPS, CgA, S-100B**
AFP (α₁ – fetoprotein)

• glycoprotein synthesized in large quantities by the fetal yolk sac and liver
• one of the major proteins in the fetal circulation

• in adults AFP /S ↑: pregnancy
  liver diseases

• marker for hepatocellular and germ-cell carcinoma
• cut off value < 13.0 IU/ml
CEA (carcinoembryonic antigen)

- family of related oncofetal cell-surface glykoproteins
- nonspecific

- ↑: liver cirrhosis, pulmonary emphysema, benign breast cysts disease, ulcerative colitis, rectal polyps lung, ovarian and breast carcinoma

- marker for colorectal, pancreatic, gastric and bile ducts Ca

- cut off value < 4.6 ng/ml
Carbohydrate markers (CA - carbohydrate antigen)

- high-molecular-weight mucins or blood group antigens on the tumor cell surface or secreted by the tumor cells

- **CA 15-3**
  - marker in monitoring of therapy and disease progression in metastatic breast cancer
  - cut off value ≤ 28 IU/ml

- **CA 125**
  - marker for ovarian and endometrial carcinomas
  - cut off value ≤ 35 IU/ml

- **CA 19-9**
  - marker for pancreatic and colorectal carcinoma
  - cut off value ≤ 37 IU/ml

- **CA 72-4**
  - marker for carcinomas of stomach, pancreas and ovary
  - cut off value ≤ 5 IU/ml
**SCC (squamous cell carcinoma antigen)**

- glycoprotein Ig receptor physiologically present in squamous epithelium cells

- ↑: pregnancy lung dis., hepatic and renal failure

- marker for pulmonary (NSCLC), orofacial, genital, and endometrial carcinoma

- cut off value ≤ 1.5 μg/l
**CYFRA 21-1 (cytokeratin fragment)**

- cytokeratin fragment present in lung, uterine and GIT cells. Marker of degradation of malign tissues and necrosis.

- ↑: cirrhosis, asthma, respiratory infections, renal failure

- marker for **cervical and pulmonary (NSLC) carcinoma**

- **cut off value** \( \leq 3,3 \mu g/l \)
MCA (mucinous carcinoma antigen), MSA (mammar serum antigen)

- ↑: breast Ca

TATI (tumor associated trypsin inhibitor)

- ↑: GIT Ca and ovarian mucinous cystadenoCa
**hCG (human chorionic gonadotropin)**

- Glycoprotein secreted by the syncytiotrophoblastic cells of placenta
- Consists of two subunits: **α-subunit** (common to several other hormones, e.g. FSH, LH or TSH)
  **β-subunit** (unique to hCG)

- ↑: pregnant women
  - hydatidiform mole

- Marker for tumors of placenta (trophoblastic tumors, particularly choriocarcinoma), and germ-cell tumors of the testis and ovary

- Cut off value < 2.00 IU/l males, < 9.00 IU/l females
**SP-1 (Schwangerschaftsprotein; β₁-specific pregnancy glycoprotein)**

- mixture of glycoproteins produced by the trophoblastic cells of placenta

- ↑: pregnant women
  - chorioCa, germinative Tu
**PSA (prostate-specific antigen)**

- glykoprotein protease (237 AA, Mr = 33 000) produced exclusively by the epithelial cells of the prostate gland, secreted into seminal fluid. In serum, it occurs as free fPSA and chymotrypsin or α₂-macroglobulin bonded.

- ↑: benign prostatic hyperplasia BPH, prostate inflammation, urological manipulations

- marker for screening (men > 50y, urinating difficulties), diagnostic and monitoring of course and treatment of prostate cancer

- cut off value < 4,0 µg/l
Derived parameters

- **tPSAD (tPSA density):** adjustment of BPH and PCa: 0,15
- **tPSA-TZ (tPSA/transition zone ratio)**
- **PSAV (tPSA velocity):** healthy 0,04 µg/l/y, BPH 0,07-0,27 µg/l/y, PCa ≥ 0,75 µg/l/y
- **age specific tPSA levels:** normal ↑ 0,04 µg/l/y; **cut off** 40-49 y 2,5 or 1,5 µg/l, 50-59 y 3,5 or 2,5 µg/l, 60-69 y 4,5 µg/l, 70-79 y 6,5 or 7,5 µg/l
- **index fPSA/tPSA:** ↓ in CaP than BPH (**cut off** < 20%)
- **tPSADT (tPSA doubling time):** adjustment of local recurrence x metastasis (shorter time) after radical prostatectomy
**NSE (neuron-specific enolase)**

- **enolase** - enzyme of glycolysis (2-phosphoglycerate → phosphoenolpyruvate)
- **NSE** - form of enolase found in neuronal and neuroendocrine tissues
- ↑: lung and liver dis., renal failure
- marker for small-cell lung cancer (SCLC), pheochromocytoma, neuroblastoma, medullary carcinoma of the thyroid, melanoma, and pancreatic endocrine tumors
- **cut off value** < 15 µg/l
**TK (thymidine kinase)**

- **enzyme of DNA synthesis** (thymidine → thymidine monophosphate)

- ↑: psoriasis, sarcoidosis, kolagenosis, rheumatic dis., pernicious and megaloblastic anemia
  
  lung, breast, prostate, testicular or colorectal Ca

- marker for **hematologic malignancies monitoring**

- **cut off value < 5 IU/l**
**LD (lactate dehydrogenase)**

- enzyme of glycolysis (lactate ↔ pyruvate)
- non-specific

- ↑: heart failure, lung and liver dis. etc. (isoenzymes)
  hematological and other malignances
  ↑ isoenzyme LD5 in liver metastases

- marker for acute leukemia, non-hodgkin lymphoma, testikular Ca and Ewing Sa monitoring

- ref. values: ♂ 3,3 - 7,5 µkat/l, ♀ 3,3 - 6,3 µkat/l
**ALP (alkaline phosphatase)**

- **Zn**\(^{2+}\) glycoprotein, in alkaline environment (pH= 8-10) it catalyses the hydrolysis of \(\text{H}_3\text{PO}_4\) monoesters and transphosphorylation

- **bone isoenzyme**
- ↑: osteoSa, bone metastases
  - other bone affections; growth

- **liver isoenzyme**
- ↑: liver metastases
  - other liver diseases

- **Regan isoenzyme (placental alike)**
- ↑: GIT and lung tumors

- **ref.values**: adults 0,5-2,4 µkat/l, 1 month - 15 years
  1,0-4,8 µkat/l, newborns 0,6-5,3 µkat/l
Cathepsins

- Lyzosomal proteinases, participate in connective tissue and cell membranes degradation → facilitate the tumor progression and origination of metastases

- **cathepsin B** ↑ in breast, ovarian, colorectal, stomach and laryngeal Ca

- **cathepsin H** ↑ in breast, lung, head and neck Ca, mainly in tumor progression and occurrence of metastases

- **cathepsin D** ↑ in adenoCa mammæ
Prolactin

- peptide (198 AA) anterior pituitary hormone that stimulates and maintains the secretion of milk. Its secretion is stimulated by TRH and inhibited by dopamine.

- ↑: prostatic and breast cancer

- marker for prolactinoma

- ref. values males 80 – 390 mIU/l
  females 70 – 520 mIU/l
**ACTH (adrenocorticotropic hormone)**

- anterior pituitary hormone that stimulates the secretion of glucocorticoid hormones. It's secretion is stimulated by corticotropin-releasing hormone of hypothalamus.

- ↑: ectopic formation: SCLC, Ca of pancreas, breast, GIT

- marker for *pituitary tumors*

- cut off value < 46.0 ng/l
**ADH (antidiuretic hormone, vasopressin)**

- **peptide (9 AA) hormone** synthesized in the supraoptic nuclei of the hypothalamus and transported via the hypophyseal tract to the posterior pituitary. It`s binding and stimulation of insertion of aquaporines into distal and collecting tubules membranes increases the water reabsorption $\rightarrow \uparrow$ blood pressure and $\downarrow$ excretion of urine

- $\uparrow$: pneumonia, porphyria

- marker for **SCLC, adenocarcinoma of the lung**

- **ref. value 2 - 8 ng/l**
**PTH (parathyroid hormone)**

- **peptide (84 AA) hormone of the parathyroid glands.** It's release is stimulated by $\text{Ca}^{++}/\text{P} \downarrow$. PTH activates osteoclasts, $\uparrow \text{Ca}^{++}$ renal resorption and $\uparrow \text{Ca}^{++}$ intestinal absorption via stimulation of calcitriol renal production $\rightarrow \uparrow \text{Ca}^{++}/\text{P}$.

- $\uparrow$: secondary and primary hyperparathyroidism

- **marker for parathyroideal tumors**

- **ref. values** $10 – 65 \text{ ng/l, } 1.6 – 6.9 \text{ pmol/l}$
**Calcitonin**

- polypeptide (32 AA) produced by the C cells of the thyroid
- normally secreted in response to $\uparrow \text{Ca}^{++}$ /P
- inhibits the release of calcium from bone (inhibition of osteoclasts)

- marker for **medullary carcinoma of the thyroid**

- cut off value $\leq 19 \text{ ng/l}$
Ferritin

- Fe$^{3+}$ storage protein

- ↑: iron overload
  - acute hepatitis and liver cells necrosis, inflammations (positive acute phase protein)

- marker for Hodgkin lymphoma and melanoma monitoring

- ref. values males 48 - 708 pmol/l, females 20 - 640 pmol/l
**β₂-microglobulin**

- part of the class 1 HLA, physiologically produced by B-lymphocytes and plasmocytes, helps Tc function

- ↑: inflammations, chronic renal and liver dis. after chemo- and radiotherapy

- marker for MM dg, CLL therapy

- ref. value 1 – 2.3 mg/l
**κ, λ light chains (paraproteins, Bence Jones proteins)**

- the first described tumor markers produced by neoplastic plasma cells in monoclonal gammopathies. They are small enough (22 kD) to pass through the kidney into the urine → prerenal „over-flow“ proteinuria.

- ↑: **monoclonal gammopathy of uncertain significance** (isolated finding of a Bence Jones protein without a clinical symptomatology)
  - Waldenström macroglobulinemia, lymphomas and leukemias, osteogenic sarcoma, bone metastases

- marker for **multiple myeloma**

- **ref. values:** FLC (free light chains)/S: κ = 3.3-19.4 mg/l, λ = 5.7-26.3 mg/l, index κ/λ = 0.26-1.65; **polyclonal FLC/U = 1-10 mg/24h**; κ/U = 1.25-5.5 mg/l, λ/U = 0.51-3.2 mg/l, index κ/λ = 0.82-3.0
**TPA (tissue polypeptide antigen)**

- non-specific cytokeratins fragments produced by both normal and tumor cells
- ↑ levels seen in increased cell proliferation → its estimation is useful for monitoring of the disease

- ↑: liver dis., DM, rheumatoid dis. breast and GIT tumors

- TPA/ U - marker for urinary bladder carcinoma

- cut off value ≤ 85 U/l
CgA (chromogranin A)

- glycoprotein of the secretory vesicles of the neuroendocrine cells. Precursor to several functional peptides including vasostatin, pancreastatin, catestatin and parastatin. **Regulates the storage and/or secretion of hormones and peptides inside the cell.**

- ↑: renal and hepatal dis., corticosteroids therapy

- marker for carcinoid, pheochromocytoma, SCLC, MEN I, neuroendocrine tumors of GIT, particularly pancreas, children’s neuroblastomas

- ref. value < 35 U/l
**S-100B**

- Ca\(^{2+}\)- binding protein of nervous system glial cells and melanoma cells. Participates on signal transmission, enzyme activity regulation and homeostasis → **stimulates neuronal growth and ↑ their survival.**

- ↑: **brain injury**
  - Down and Alzheimer dis.
  - **malignant melanoma**

- marker for **malignant melanoma monitoring**

- ref. value < 0,105 µg/l
New bone markers in bone metastases dg and monitoring

- **P1NP (N-terminal propeptide of type I collagen):** monitoring of bone formation, highly specific for osteoblastic metastases formation. Is released into IC environment during collagen synthesis, than into the blood → assessment /S.

- **b-CTX (β-Cross Laps):** bone resorption marker, monitoring of antiresorption therapy
- **ref. value < 0,704 µg/l**

- **ICTP (c-telopeptide of type I collagen):** marker of bone resorption mediated by MMP9 (matrix metaloproteinase 9), activated in pathological conditions (physiological bone resorption is mediated by cathepsin K). Replaced by b-CTX.
Other perspective prognostic and predictive markers

- **MMP-7 (matrilysin):** metalloproteinase participating in EC matrix degradation, tumor invasion and progression, used in *breast Ca*

- **SMR (mesomark, soluble mesothelin-related protein):** marker for *mesothelioma* (no marker before now)

- **Heparanase:** cleavage of heparane sulphate. ↑ metastatic potential of tumor, ↓ surgical survival; for example in *pancreatic Ca*.

- **RECAF (AFP receptor):** allows transport of molecules into the fetal and malignant cells. Detectable in the tissues of *breast, ovarian, lung, stomach, prostate, and cervical Ca, lymphoma, melanoma* etc.

- **hK (human kallikreins):** Ser proteases of hormone-dependent tissues, used in *prostate and ovarian Ca; hK3 = PSA*