TUMOR MARKERS

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USE 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**

Tu markers kinetics

- **Doubling time** = time to double its (serum/plasma) level. **The shorter, the more aggressive** Tu growth.

- **biological half-life:**

Marker	Days	Hours	Marker	Days	Hours
PSA	2		NSE	1	
CA 19-9	5		CYFRA 21-1		3
CA 125	4		CEA	14	
CA 15-3	7		TPA	7	

CLASSIFICATION OF TUMOR MARKERS

- **According to proof:** humoral, cellular
- **According to chemical structure** (glykoproteins, glykolipids, polypeptides, imunoglobulins, polyamines)
- **According to visceral specificity**
- **According to** physiological **function** (oncofetal antigens, oncoplacental antigens, enzymes, hormones, serum proteins, receptors and others)

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

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.

- **CEA**
- **CA (carbohydrate antigens)**
- **AFP**

- **SCC** (squamous cell carcinoma)
- **MCA** (mucinous carcinoma antigen)
- **MSA** (mammary serum antigen)
- **TATI** (tumor associated trypsin inhibitor)

CEA (carcinoembryonic antigen)

- **family of related oncofetal cell-surface glycoproteins**
- nonspecific

- ↑: liver cirrhosis, pulmonary emphysema, benign breast cysts disease, ulcerative colitis, rectal polyps
  colorectal, lung, ovarial, pancreatic, gastric and bile ducts Ca

- **marker for colorectal and breast carcinoma, pancreatic, gastric and bile ducts Ca**

- **cut off value < 5.0 ng/ml**

CEA – 1st choise marker of colorectal Ca (CRCA

- **One of the most common malignancies in both sexes** in economically developed countries
- Incidence has increased more than 3 times during last 30 years.
- Prevalence is increasing annually by 2–3%.
- CR – newly diagnosed around 8 000 patients per year and about half of them die from CRCA.
- CR – 3rd most common malignancy in ♂, 4th in ♀
- 25 % is diagnosed metastasized!

2017 data.

Colorectal carcinoma

- possibilities of prevention :

Primary
Lifestyle, nutrition

Secondary
Broadcast screening from 1.7.2000 (CR)-
cyclic fecal occult blood testing in asymptomatic individuals from age 50
or
screening colonoscopy from age 55.

CRCA – other possibilities of detection **at an early stage**

- **detection of early adenoma lesions or initial phases of CRCA:**

a) detection of mutations (PCR, DNA biochips) in faeces and blood:

Point mutations *KRAS*, *APC;* mutations of instability markers *BAT 26*, *P53; DNA* integrity test

a) Detection of new biomarkers:

In faeces:

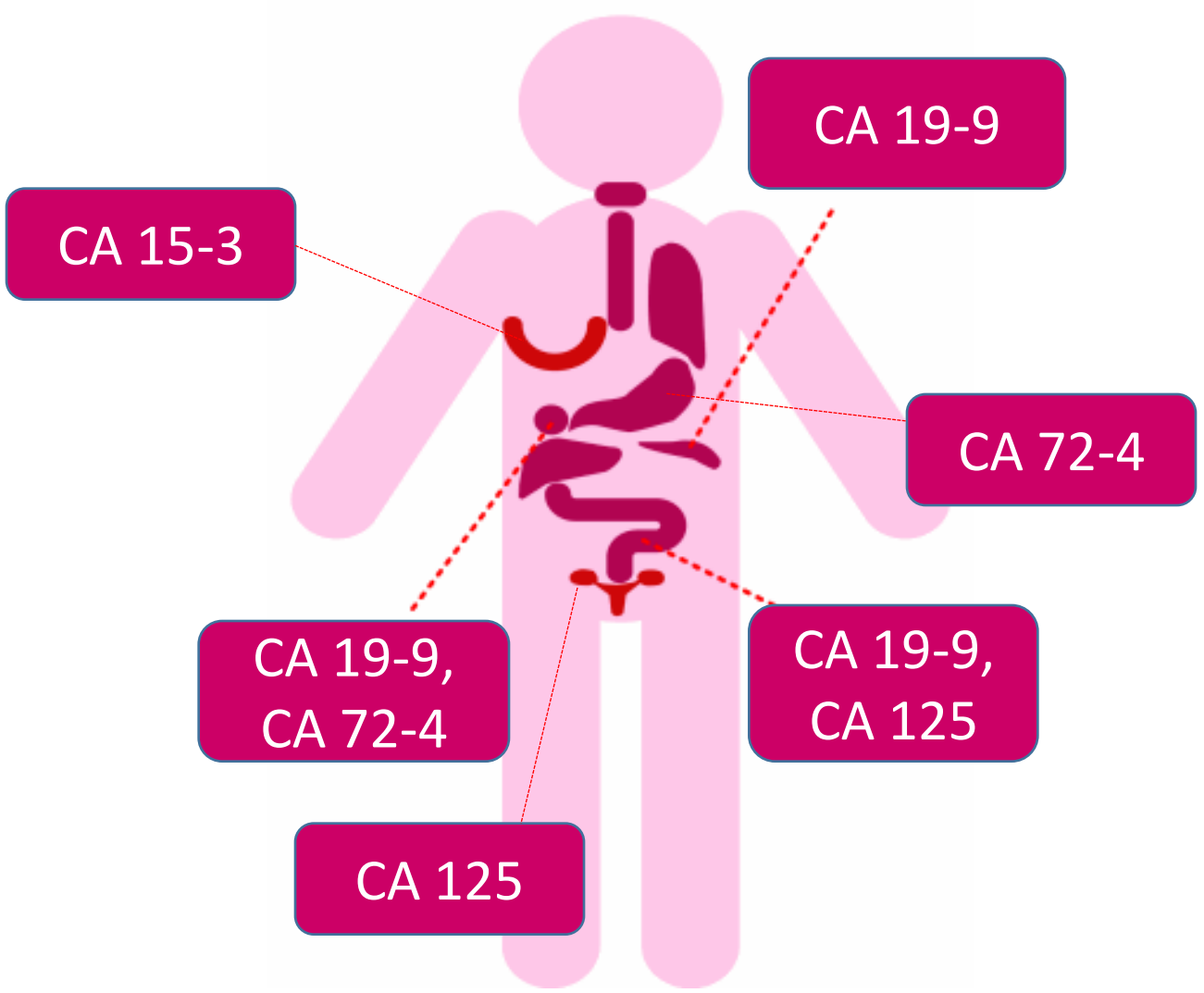
CNRIP1, INA, MAL, SNCA, SPG20

In blood:

matrix metaloproteinase 2 (MMP 2),
tissue inhibitor of metaloproteinase 2 (TIMP 2)
Antibodies against RPH3AL *(rabphilin 3A-like protein)*
Monitoring of serum cytokines or HLA-G

CA (carboydrate antigens)

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



CA 72-4 (carbohydrate antigen 72-4)

- glycoprotein produced by oesophageal, gastric and pancreatic epithelium
- in adults ↑: liver diseases, acute pancreatitis, gastric ulcer, inflammations of GIT
  Ca of stomach, colon, uterus, lung (NSCLC)
- **marker for monitoring of gastric Ca (1st choice marker), pancreatic, oesophageal and ovarian Ca**
- **cut off ≤ 7 IU/ml**

CA 19-9 (carbohydrate antigen 19-9)

- glycoprotein of fetal GIT, pancreas and liver epithelium; in adults it is produced by GIT and bronchial epithelium.
- **marker for pancreatic, colorectal and gastric carcinoma**
- **cut off value ≤ 40 IU/ml**

CA 19-9

- **Sensitivity** in selected Tu (Klinická biochemie a metabolismus, 2009)

Ca	Sensitivity / %
pancreatic	70-90
colorectal	18-58
cholangiocellular	22-49
bile ducts	55-79
gastric	25-60

CA 125 (carbohydrate antigen 125)

- glycoprotein of airways and digestive tract epithelium of both fetuses and adults

- ↑: ***ovarial, colorectal Ca**

  * endometrial, breast, pancreatic, liver and pulmonary Ca
  * pregnancy, breast milk
  * benign diseases of ovaries and endometrium, hepatitis, icterus, pancreatitis

- **marker for dg and monitoring of therapy of non-mucinous ovarian Ca; additional marker for pancreatic and colorectal Ca**

- **cut off ≤ 35 IU/ml**

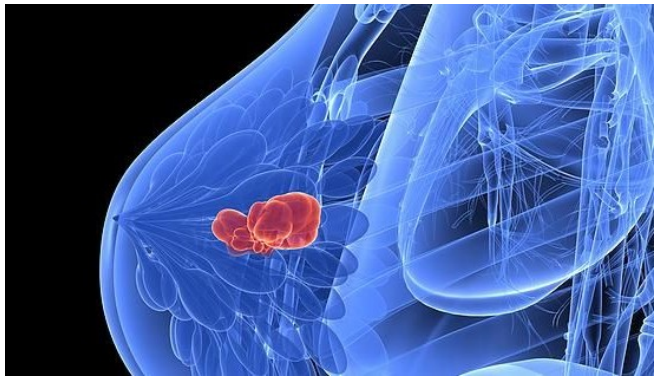
CA 15-3 (carbohydrate antigen 15-3)

- glycoprotein of fetal bronchial and hepatic cells, adult mammary cells

- **in adults ↑: pregnancy**
  rheumatic dis., chronic dis. of liver, stomach, pancreas, ovaries, uterus, prostatic gland, AIDS
  Ca of organs mentioned above
-
- **marker for breast Ca monitoring**

- **cut off ≤ 35 IU/ml**

CA 15-3 and CEA – 1st choice markers for breast carcinoma



- **The 2nd most common Ca in females**
- Incidence – 1 million of woman worldwide
- 90 - 95% sporadic
- 5 – 10% inherited - BCRA1 and 2 gene mutations – possibility of DNA testing from peripheral lymphocytes
- The lifetime risk of developing cancer for BRCA1/2 is 87%, in women without mutation 8-10%.

- Secondary prevention –mammography or ultrasound examination from 45 years of age

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 < 10 μg/l**

AFP (α1-fetoprotein)

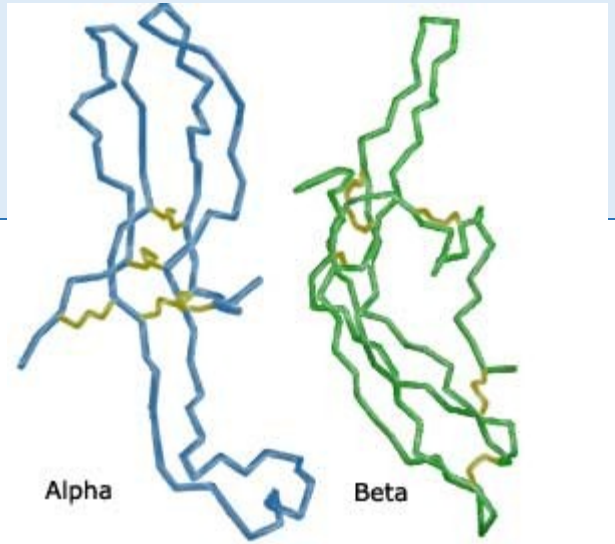
- **Sensitivity in selected Tu** (Klinická biochemie a metabolismus, 2009)

Tumor	Sensitivity / %
Hepatocellular Ca	80
Embryonal Tu	80
Teratoma	20
Yolk sac Tu	80

Oncoplacental antigens

- Substances 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

- **hCG**



hCG subunits

- **SP-1**

hCG (human chorionic gonadotropin)

- glycoprotein secreted by the syncytiotrophoblastic cells of the 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, < 10.00 IU/l females** (βhCG)

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

• **PSA** • **ALP**	• **NSE (neuron specific enolase)** • **TK (thymidinkinase)** • **LD** • **kathepsins**

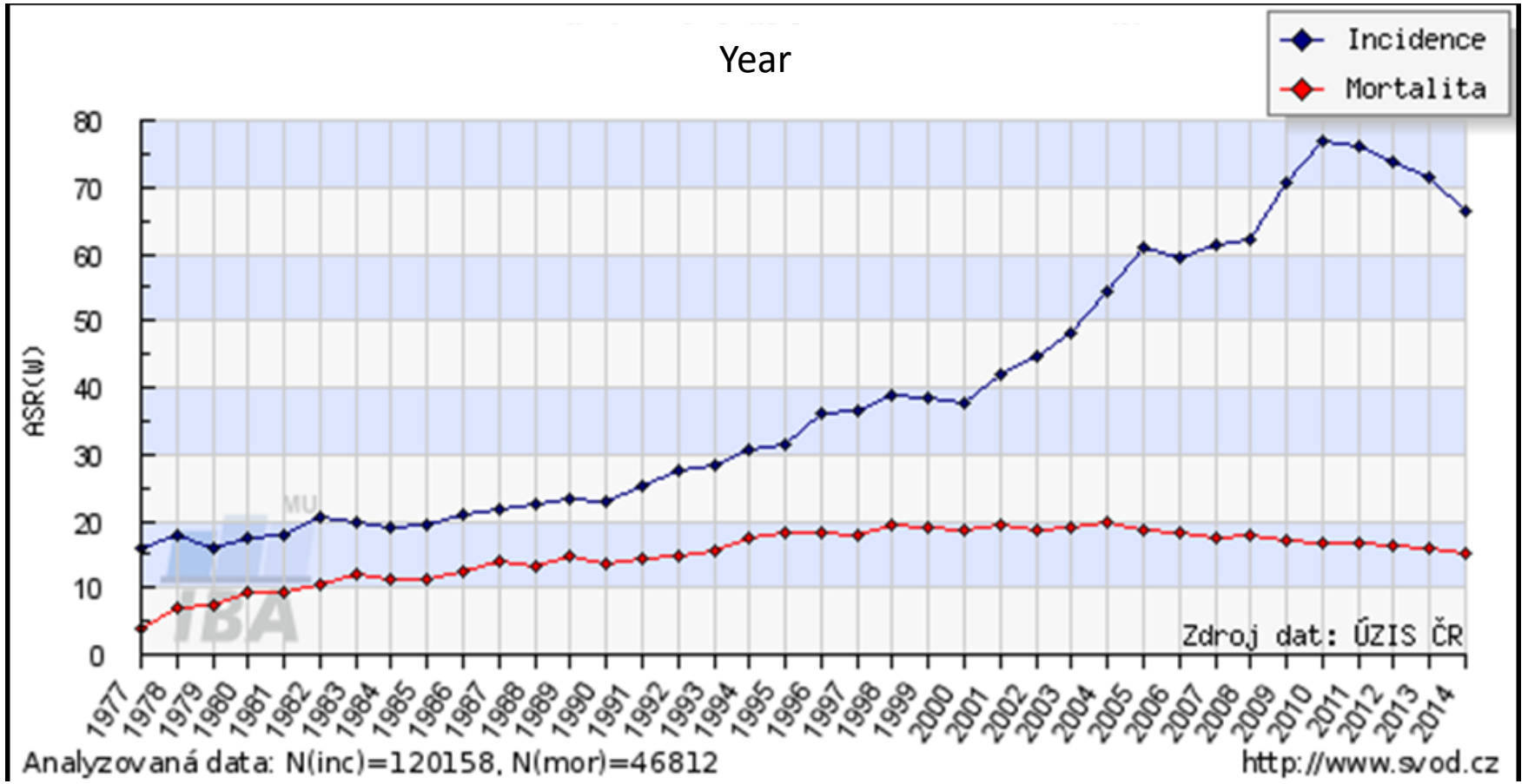
Prostate carcinoma

- 2nd most common malignancy in men, CR
- 7 049 new cases in 2015 (136 new cases/ 100 000 males)
- Risk factors:

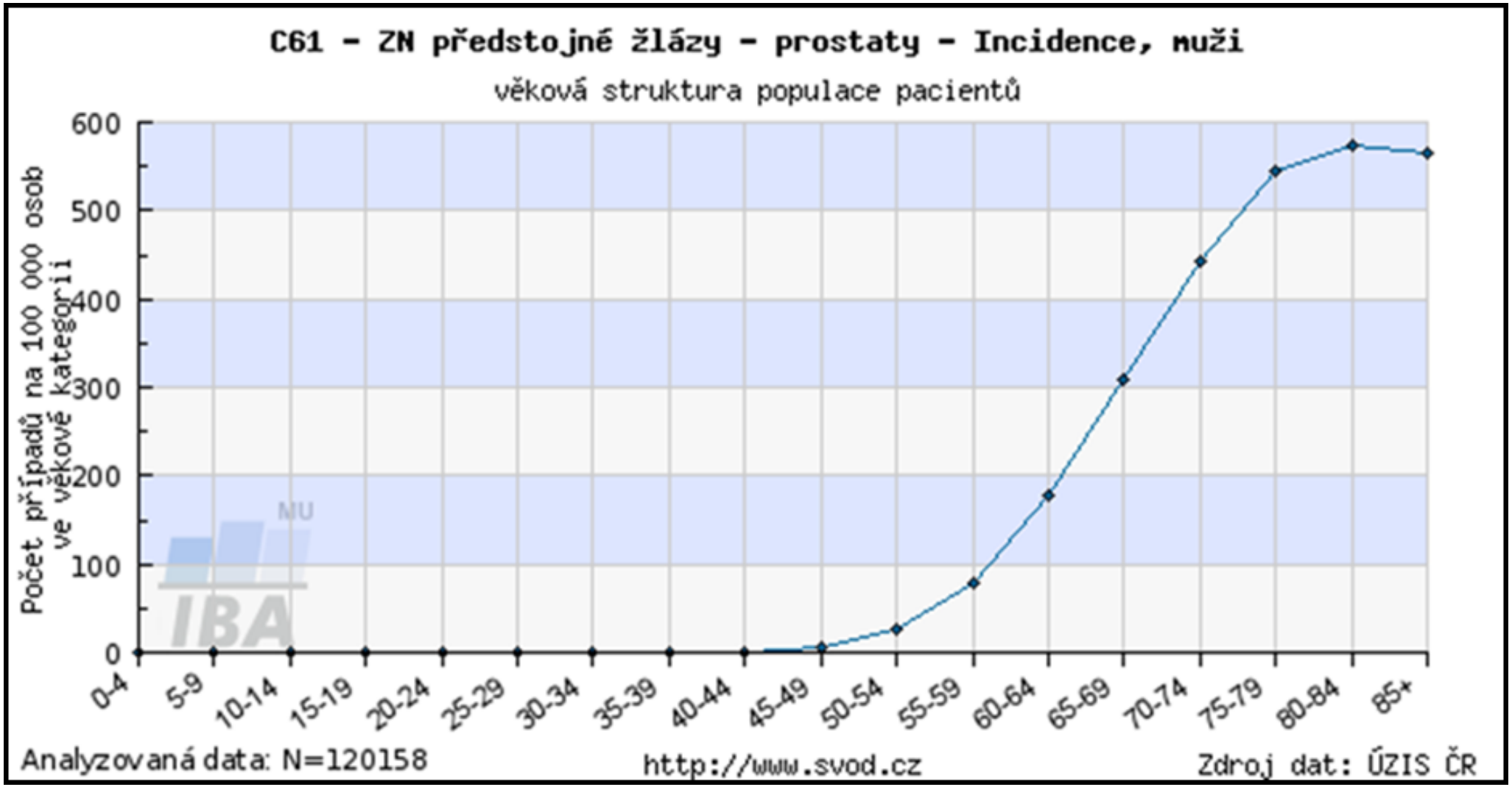
  age,

  life style,

  genetic factors



Epidemiology of prostate cancer



PSA (prostate-specific antigen)

- glykoprotein **protease** (237 AA, Mr = 33 000) **produced exlusively by the epitelial cells of the prostate gland, secreted into seminal fluid** (liquefaction).
- Produced as inactive proPSA → PSA.
- **In serum,** it occurs **as free fPSA and** α_1-antichymotrypsin or α_2-macroglobulin **bound** (55-95%).

- **↑: benign prostatic hyperplasia BPH, prostate infammation, urological manipulations**

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

- **cut off value < 4.0 µg/l (= ng/ml) (> 50 y), 2.5 µg/l (< 50 y,** see more in ***age specific levels***)

Increased levels of total PSA in plasma / serum

- **age specific levels:**

cut off 40-49 y. 2,5 ng/ml, 50-59 y. 3,5 ng/ml,

60-69 y. 4,5 ng/ml, 70 and more y.6,5 ng/ml

- tPSA > 10 ng/ml: suspicious PCa, we perform another examinations
- tPSA 4 – 10 ng/ml: PCax BHP???, we perform another examinations

Derived parameters

- ***index f/t PSA – free/total PSA:*** fPSA< 15%: probable PCa,
  fPSA > 20% probable benign condition
- ***tPSAD (tPSA density):***
- ratio [tPSA]/$_{UTS}$ prostate volume in cm^3
- adjustment of BPH and PCa: cut off 0.15 ng/ml
- ***PSAV (tPSA velocity):***
- increase of [tPSA] / year
- healthy 0.04 ng/ml/y, BPH 0.07-0.27 ng/ml/y, PCa ≥ 0.75 ng/ml/y
- ***tPSA doubling time:***
- time to double [tPSA]
- ***tPSA-TZ:***
- [tPSA] / transition zone volume

Other derived parameters

- proPSA
  - isoforms **(-2)proPSA** and (-4)proPSA typical for PCa, clinical significance(-2)proPSA

- PHI (Prostate health index)
  - $PHI = \frac{(-2)proPSA}{fPSA} . \sqrt{tPSA}$
  - higher specificity than fPSA/tPSA

Other causes of PSA increase in blood

- **Other prostate diseases:** benign prostate hyperplasia, prostatic inflammation
- **Mechanical stimulation** (fPSA is more susceptible): biopsy, cystoscopy, catetrization, per rectum examination
- Ejaculation
- PSA is a prostate-specific biochemical marker but is not specific for cancer.

ALP (alkaline phosphatase)

- **Zn^{2+} glycoprotein, in alkaline environment (pH= 8-10) it catalyses the hydrolysis of H_3PO_4 monoesters and transphosphorylation**

- **bone isoenzyme (b-ALP)**
- **↑: osteoSa, bone metastases**
  other bone affections; growth
- **liver isoenzyme (l-ALP)**
- **↑: liver metastases**
  other liver diseases

- **ref.values : adults 0.5-2.15 μkat/l, 1 month - 15 years 1.35-7.5 μkat/l,**
  newborns 1.2-6.3 μkat/l

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,** ectopicaly produced by the **lung small cells)**
   elevation of a hormone is not specific ← it may be produced by a variety of cancers

• **prolactin** • **calcitonin** • **PTH** (1.)	• **ACTH** • **ADH** (2.)

Serum proteins

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

- paraproteins

- ferritin
- β_2-microglobulin

Monoclonal immunoglobulins (paraproteins)

- **the first described tumor markers produced by neoplastic plasma cells in monoclonal gammopathies. In serum, we can identify whole Ig, heavy chains (IgG, M, A; D, E) and *κ, λ light chains (Bence Jones proteins)* -** these are small enough (22 kD) to pass through the kidney into the urine → **prerenal „over-flow“ proteinuria.**

- ↑: **multiple myeloma and other monoclonal gammapathies,** lymphomas and leukemias, osteogenic sarcoma, bone metastases

- **marker for multiple myeloma and other monoclonal gammapathies**

- **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; paraprotein/U - obsolete

Receptors

Cellular (tissue) markers used in hormone-producing tumors	
• Estrogen rec. • Progesterone rec.	• Growth factors receptors (HER1, HER2/neu) • DNA aneuploidy

The main usage: breast Ca, colorectal Ca; brain tumors

Estrogen and progesterone receptors

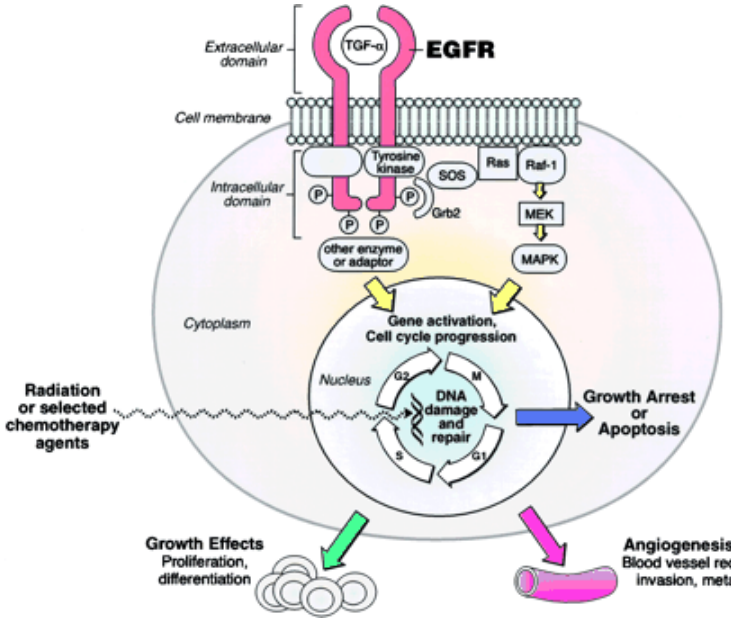
- **The most important prognostic markers for breast Ca;** detected in tumor tissue.

- **positivity =** ↑ cell diferentiation, ↓ invasivity, **better prognosis;** = antiestrogen therapy indication

- **immunohistochemical determination: positivity % value**
- **ELISA: cut off < 15 fmol/mg of protein**

Growth factors receptors

- Transmembrane receptors with tyrosinkinase activity – phosphorylation of Tyr fosforylují tyr residues of protein substrates
- The binding of substrate to the extracellular domain causes a conformational change of the receptor, its autophosphorylation and activation of downstream signaling pathways → influence of cel. proliferation, inhibition of apoptosis
- **HER1 (EGFR), HER/2neu,** HER3, HER4



Growth factors receptors

Type of receptor	HER1	HER2/neu*
Ligands	EGF, TGFα, amphiregulin, betacelulin, epigene, epiregulin	?
Possibility of blocking	Monoclonal AB (cetuximab, erlotinib,...)	Monoclonal AB (trastuzumab)

* Nomenclature: HER 2 in humans, neu in rodents

Breast carcinoma markers - summary

- Basic markers: CEA and CA 15-3
- Receptor markers:
  - Growth factors receptors
  - Estrogene receptor
  - Progesterone receptor
- Markers of metastasis and proliferation - see further

Other tumor markers

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

• TPA, TPS • Mesotelin	• Chromogranin A • Neuropeptide Y • S-100 β • 5-hydroxyindolacetic acid

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

- **marker for urinary bladder carcinoma**

- cut off value ≤ 140 IU/l

Tumor markers – lungs, bronchi, trachea

Non-parvicellular Ca (NSCLC)

CEA

CYFRA 21-1

Parvicellular Ca (SCLC)

NSE

CYFRA 21 -1

CYFRA 21-1 (cytokeratin fragment)

- **Cytokeratin 19 fragment present in lung, uterine and GIT cells. Marker of degradation of malignant tissues and necrosis.**
- **↑: cirrhosis, asthma, respiratory infections, renal failure**
- **marker for cervical and pulmonary (NSCLC) carcinoma**
- **cut off value ≤ 3.3 μg/l**

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**

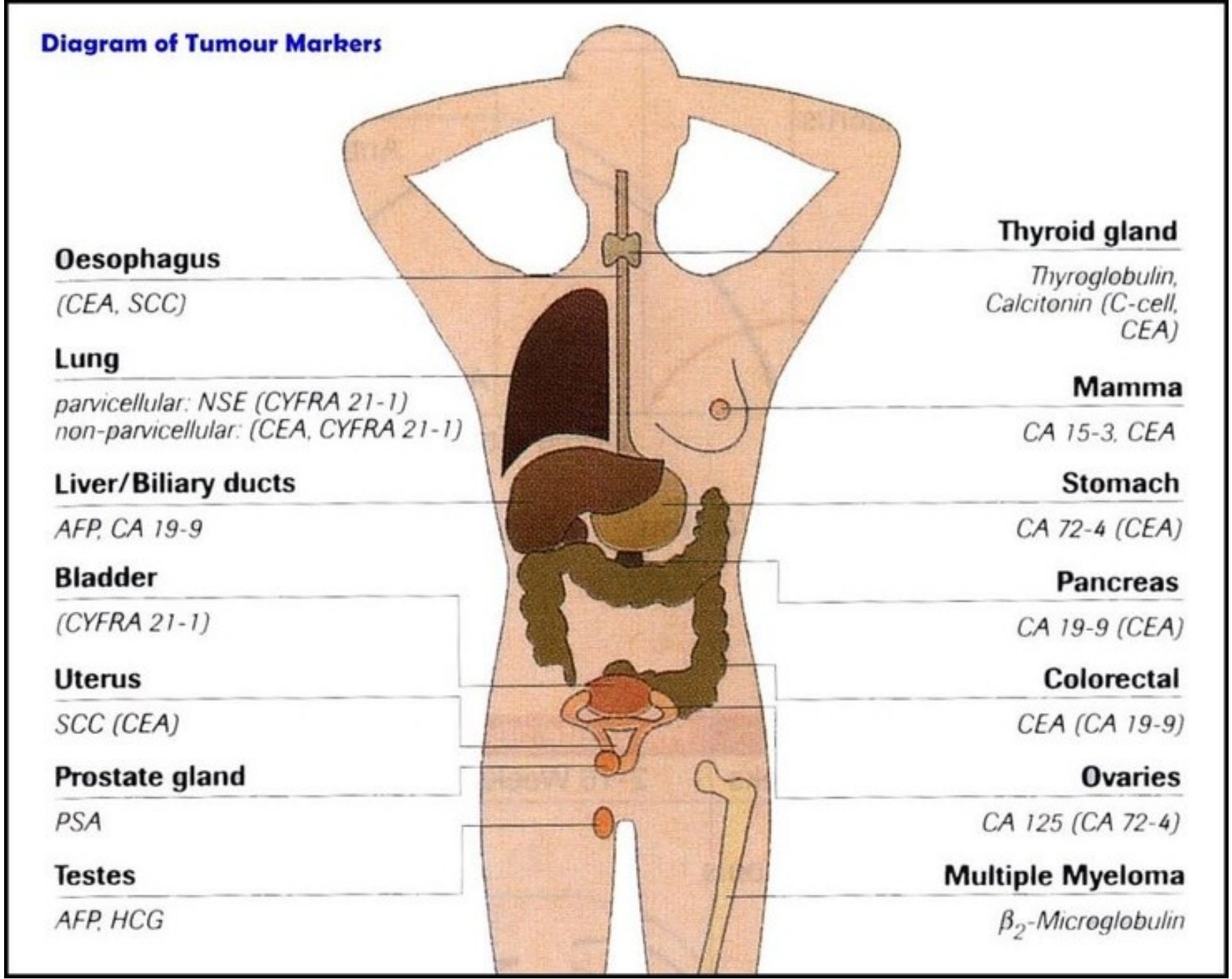


Diagram of Tumour Markers
Oesophagus
(CEA, SCC)
Lung
parvicellular: NSE (CYFRA 21-1)
non-parvicellular: (CEA, CYFRA 21-1)
Liver/Biliary ducts
AFP, CA 19-9
Bladder
(CYFRA 21-1)
Uterus
SCC (CEA)
Prostate gland
PSA
Testes
AFP, HCG
Thyroid gland
Thyroglobulin, Calcitonin (C-cell, CEA)
Mamma
CA 15-3, CEA
Stomach
CA 72-4 (CEA)
Pancreas
CA 19-9 (CEA)
Colorectal
CEA (CA 19-9)
Ovaries
CA 125 (CA 72-4)
Multiple Myeloma
β_2-Microglobulin

Markers for dg and monitoring of bone metastasis

Bone metastases: tumors of lungs, prostate, breast

Monoclonal gamapathies

New bone formation markers

Usage: monitoring the effect of treatment on osteoblastic metastases

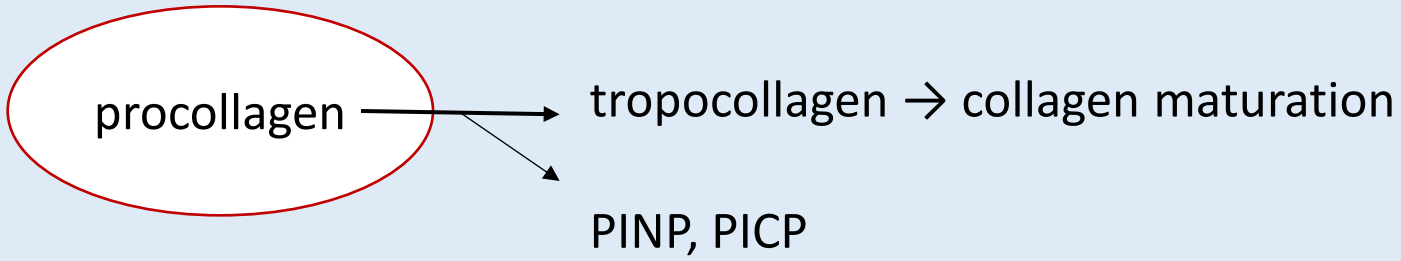
Bone resorption markers

Usage: dg bone mass distribution of solid tumors (PCa), monitoring the effect of antiresorptive treatment

Markers in bone metastases

Bone formation markers

- **PINP (N-terminal propeptide of type I procollagen)**



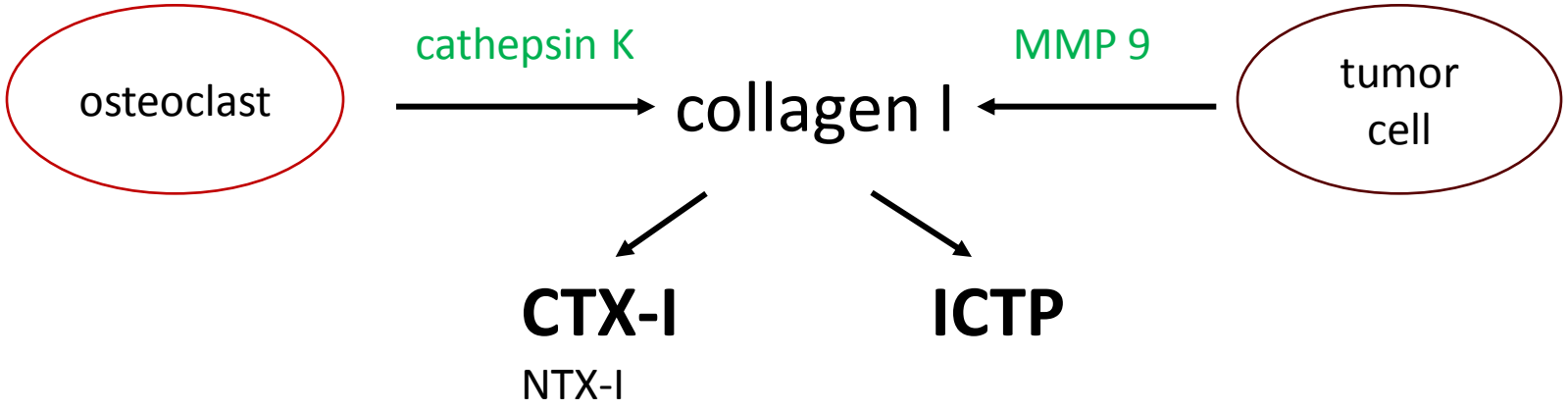
- **Osteocalcin -** serum levels are proportional to its formation in osteoblasts.

- **Bone ALP –** bone izoenzyme of ALP, serum levels are proportional to osteoblasts activity

Markers in bone metastases

Bone resorption markers

- **ICTP (C-telopeptide of type I collagen):** marker of collagen degradation by action of MMP 9
- **CTX-I (β-CTX β-Cross Laps, C-terminal telopeptide of type I collagen):** marker of collagen degradation by action of enzymes from osteoclasts



Proliferative antigen Ki-67

- The non-histone nuclear protein expressed during active cell cycle phases (max at the G2 interface and mitosis, is absent in the G0 phase).
- It affects the spatial layout of chromatin - gene expression control.
- Immunohistochemistry detection in biopsy tissue - Anti-Ki-67 antibody.
- Ki-67 expression = proliferative tumor activity.
- Proliferative activity in cancer correlates with grade and prognosis.

= prognostic marker determined in tumor tissue of solid tumors